



CENTER FOR BIOENVIRONMENTAL RESEARCH

at Tulane and Xavier Universities

Office of Naval Research Annual Productivity Report: 2003-2004

ONR N00014-99-1-0763



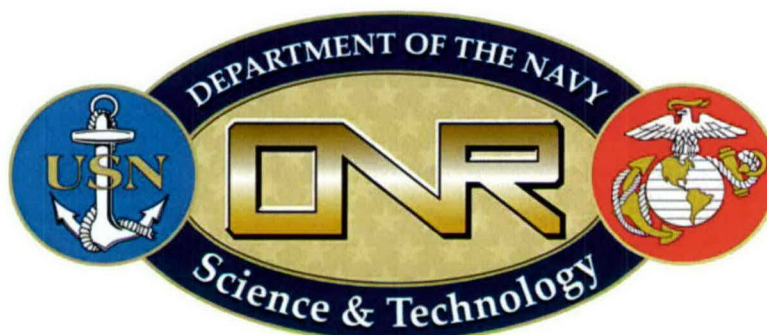


CENTER FOR BIOENVIRONMENTAL RESEARCH

at Tulane and Xavier Universities

Integrated Bioenvironmental Hazards Research Program

US Department of the Navy
Office of Naval Research



Productivity Report, 2003 - 2004
N00014-99-1-0763

DISTRIBUTION STATEMENT A
Approved for Public Release
Distribution Unlimited

N00014-99-1-0763
INTEGRATED BIOENVIRONMENTAL HAZARDS RESEARCH PROGRAM
ANNUAL PRODUCTIVITY REPORT
TABLE OF CONTENTS

Abstract

List of Acronyms and Abbreviations

Annual Productivity Report

- I. Objectives and Significance
 - A. Environmental Priorities of the Office of Naval Research (ONR)
 - B. Prior Progress of the CBR Bioenvironmental Hazards Research Program
 - C. Current Research Efforts of CBR Bioenvironmental Hazards Research

- II. CBR Capacity
 - A. Introduction to the CBR
 - B. The CBR Tulane/Xavier Partnership – A Unique Model
 - 1. Tulane Capabilities
 - 2. Xavier Capabilities
 - C. CBR Support Cores
 - 1. Research Support
 - 2. Computer Operations
 - 3. Environmental Informatics
 - 4. Communication and Education

- III. Overview of Research
 - A. Introduction
 - B. Environmental Signals and Sensors
 - C. Ecosystem Monitoring and Assessment

- IV. Summary Accomplishments
 - A. Overview
 - B. Environmental Signals and Sensors
 - C. Ecosystem Monitoring and Assessment

Appendices

- A. Investigative Research Reports
- B. Publications & Presentations
- C. Useable Technologies
- D. Intellectual Development
- E. Historical Documents
 - 1. BAA
 - 2. Award/Modification Letters
 - 3. Timeline

SF 298 Cover Sheet

Center for Bioenvironmental Research At Tulane and Xavier Universities
Bioenvironmental Hazards Research Program
Office of Naval Research/US Department of Defense
Annual Productivity Report
N00014-99-1-0763

ABSTRACT

Beginning in April 1999, the Center for Bioenvironmental Research (CBR) at Tulane and Xavier Universities has received funding from the Office of Naval Research to continue its Bioenvironmental Hazards Research Program (BHRP). This funding has supported a suite of complementary research projects that address the impacts of bioenvironmental hazards on environmental signaling from molecular to ecosystem levels and makes connections between these impacts. The research ranges from basic research on proteomics to applied technology development of biosensors and autonomous underwater vehicles for monitoring. The BHRP program includes mechanisms for the effective communication of this information for resolution of Department of Defense problems and for the educational training of future scientists.

One module, Environmental Signals and Sensors, utilizes basic research on how chemical signals on molecular, cellular, and organismal levels can be utilized for assessments of human, wildlife, and plant health; and development of biosensors for assessments of toxicity and risk. Areas of focus, including human and ecological health, integrate research themes in this module by extending environmental signaling to human health endpoints at individual and population levels, or extending to ecological and ecosystem function levels. Given the CBR's and Navy's mutual interest in biosensors and brown water/ocean systems, a second module, Ecosystem Monitoring and Assessment, has placed special research emphasis on the small scale turbulence of the model ecosystem of the Mississippi River/Gulf of Mexico and the development of biosensors and autonomous platforms for ONR and DOD applications. As a suite of integrated modules, specific projects thus have complemented each other as part of a holistic BHRP to aid in effective and comprehensive environmental assessments for the DOD.

Six research projects have been conducted in the two primary research modules and have resulted in significant progress related to the overall grant objectives. Assisting in the implementation of the overall project to promote the dual resolution of DOD problems and education of students and the general public was the continuation of four support cores: 1) environmental informatics; 2) computer operations; 3) research support; and 4) communication and education. In addition to the new knowledge developed by the research effort, the program has produced 2 useable technologies for further development, 22 publications and abstracts, 11 presentations, and supported the intellectual development of 4 graduate students, 8 undergraduates, and 6 CBR SPRITE students.

This program reflects the CBR's existing research strengths and employs a set of complementary, integrated research modules to assess the impacts of "environmental signals" (e.g., contaminants and pollutants) on humans and ecosystems. The integration of all the research modules has resulted in a comprehensive program of environmental research that provides the ONR with a technology package that spans research initiation to communication of environmental findings to appropriate target audiences. Transcending traditional structures, the CBR has become a model of academic/government/industry interaction.

List of Acronyms and Abbreviations

ARNT	aryl hydrocarbon receptor nuclear translocator
AUV	autonomous underwater vehicles
BHRP	Bioenvironmental Hazards Research Program
CBR	Center for Bioenvironmental Research
CdS	Cadmium sulfide
CRC	cyclicAMP response element
DES	diethylstilbestrol
EDCs	endocrine disrupting chemicals
EIC	Environmental Informatics Core
ER	estrogen receptor
ERE	estrogen response elements
ESN	Environmental Signaling Network
GIS	Geographical Information System
HMGA	High Mobility Group proteins
HIF	Hypoxia Inducible Factor
IT	Information Technology
MCB	Molecular and Cell Biology
MAPK	mitogen-activated protein kinase
NSF	National Science Foundaton
RTG-2	rainbow trout gonads
RTH-129	rainbow trout hepatocytes
RT-PCR	real time polymerase chain reaction
SPRITE	<u>S</u> ummer <u>P</u> ipeline <u>R</u> esearch <u>I</u> nitiative: the <u>T</u> ulane <u>E</u> xperience
TUHSC	Tulane University Health Sciences Center
XU	Xavier University

**Center for Bioenvironmental Research
At Tulane and Xavier Universities**

**Bioenvironmental Hazards Research Program
Office of Naval Research/US Department of Defense**

**Annual Productivity Report
July 1, 2003 – June 30, 2004
N00014-99-1-0763**

I. OBJECTIVES AND SIGNIFICANCE

The Office of Naval Research (ONR) is interested in long-range science and technology research projects that offer potential for advancement and improvement of Navy and Marine Corps operations and encourages participation by Historically Black Colleges or Universities (HBCUs). This final technical report describes an integrated program of basic and applied bioenvironmental research for technology development, communication and education that supports the ONR Bioenvironmental Hazards Research Program (BHRP). The work described in this report builds upon the DOD's fourteen-year, integrated research program (BHRP) conducted at the Center for Bioenvironmental Research (CBR) at Tulane and Xavier Universities. The CBR focuses on the holistic concept of environmental signaling from molecular to ecosystem levels, with a particular emphasis on development of biosensors, biomarkers, and evaluation techniques related to environmental exposures of human and ecological systems that addresses bioenvironmental problems relevant to the Navy and the Department of Defense (DOD). CBR research taps the basic and applied strengths of two universities that have been directed and refined over a fourteen-year period to reflect and, indeed, anticipate DOD environmental research interests.

A. Environmental Priorities of the Office of Naval Research

Many of the DOD environmental programs seek to understand the fate and biological effects of pollutants and contaminants resulting from military operations and training. To achieve this goal, DOD has focused on basic research to understand the biological actions of these agents, biomarkers of exposure, mechanisms of toxicity, and the use of experimental and computational modeling to assess potential health risks. The ONR focuses on "brown water" problems of particular Navy relevance (e.g., major world rivers, estuaries, and continental shelves). Of particular interest to the ONR Biomolecular and Biosystems Science and Technology Group are 1) biomolecular projects related to biosensor development; and 2) biosystems projects related to marine mammal biology and environmental microbiology/engineering.

Central to meeting these challenges is the continued development of new knowledge, technology, and human resources through the nation's universities, where approximately half of the defense science and technology research is currently performed. The ONR is committed to strengthening the scientific capability of colleges and universities with significant enrollments from minorities underrepresented in science and engineering, and providing science-related infrastructure as well as funding for defense research and

engineering programs. Support of the nation's university-based science and education enterprise is an essential component in addressing environmental concerns facing DOD. The CBR submitted a proposal in response to the Broad Agency Announcement (BAA 03-001). In January 2003, the Office of Naval Research awarded the Center for Bioenvironmental Research (CBR) at Tulane and Xavier Universities a one-year renewal grant of \$1,100,000 in funds for the Bioenvironmental Hazards Research Program (BHRP), beginning in July 1, 2003, through June 30, 2004. This funding has supported a suite of complementary research projects that address the impacts of bioenvironmental hazards on environmental signaling from molecular to ecosystem levels and makes connections between these impacts. The research ranges from basic research on proteomics to applied technology development of biosensors and autonomous underwater vehicles for monitoring. This annual productivity report also includes mechanisms for the effective communication of this information for resolution of DOD problems and for the educational training of future scientists.

B. Prior Progress of the CBR Biohazards Research Program

The CBR BHRP to date has developed an integrated approach to increasing the knowledge base of actual and potential impacts on human health and ecological systems of defense-related operations, as well as the processes to restore contaminated environments. To facilitate the long-term security of the Navy, other military services, and the nation, the CBR BHRP has:

- Produced a vast suite of technologies and methods for biohazard monitoring and characterization of ecosystem, wildlife, and human health for application biohazard risk assessments for all of the DOD's branches;
- Developed one of the world's most robust programs for biosensor and biomarker technology development for real-time cost-effective monitoring of heavy metal and organic contaminants and combat-related biohazards in the air, water, and the soil including one deployed on an autonomous underwater vehicle (AUV);
- Facilitated a strategic partnership between academia, military, agency, and the commercial sector in the Gulf South region for a holistic long-term monitoring program for the Mississippi River, the Gulf of Mexico, and airshed to serve as a national testing laboratory for military biohazard monitoring, characterization, and communication;
- Increased the nationwide representation of African Americans with advanced degrees in bioenvironmental fields important to DOD with increased placement of these students in NRL laboratories; and
- Funded more than 100 projects over the past decade, including those through the ONR, resulting in hundreds of publications, collaborations, and investments with the commercial sector.

These projects are being conducted through a wide variety of methods including *in vitro*, *in vivo*, epidemiological, modeling, field, and other laboratory studies at Tulane and Xavier Universities and associated sub-contractual institutions. The significance of this research includes a greater understanding of human and ecosystem responses to environmental contamination and their ability to repair or reverse these effects; increased safety for defense

workers and the general public from exposure to toxic exposures; and advanced monitoring technologies of the environment for the improvement of human and ecosystem health.

An additional benefit of the CBR BHRP is the education and research training provided to minorities. Past research activities have trained both minority faculty and students at Xavier University in scientific analytical techniques and have increased minority representation in these fields. These are techniques applicable to a variety of environmental concerns, but of particular relevance to ONR problems.

The most important result of the CBR BHRP is the development and practical application of basic knowledge and new technologies. BHRP technologies have been demonstrated through numerous environmental applications in both defense and private sectors.

Through its research, the CBR has attracted corporate investments including: \$1 million from Shell, \$75,000 from TRW, and other investments from Uniroyal and Exxon. Many of these BHRP projects have demonstrated either existing or potential commercial and civilian interest or partnerships. The useable technologies that have demonstrated the greatest commercial and civilian interest to date are summarized in **Appendix C**.

C. Current Research Efforts of CBR Biohazards Research Program

This program reflects the existing research strengths of the CBR and employs a set of complementary, integrated research modules to assess the impacts of environmental signals (e.g., contaminants and pollutants) on humans and ecosystems. The integration of all the research modules has resulted in a comprehensive program of environmental research that provides the ONR with a technology package that spans research initiation to communication of environmental findings to appropriate target audiences. There are few, if any, academic organizations with this capability. The CBR BHRP research facilitates biomarker, human and ecological health assessment, and biosensor applications to systems that provide useful models for the DOD and ONR.

One module, Environmental Signals and Sensors, utilizes basic research to study how chemical signals on molecular, cellular, and organismal levels can be utilized for assessments of human, wildlife, and plant health; and how biosensors can be developed and utilized for assessments of toxicity and risk. Areas of focus, including human and ecological health, integrate research themes in this module by extending environmental signaling to human health endpoints at individual and population levels, or to ecological and ecosystem function levels. Given the CBR's and Navy's mutual interest in biosensors and brown water/ocean systems, a second module, Ecosystem Monitoring and Assessment, has placed special research emphasis on model ecosystem study of the Mississippi River/ Gulf of Mexico and also the development of biosensors and autonomous underwater platforms for ONR and DOD applications. Cores that provide research support include computer operations, environmental informatics, and communication and education to promote the dual resolution of both DOD problems and the education of students and the general public. As a suite of integrated modules, specific projects thus have complemented each other over time as part of a holistic BHRP to aid in effective and comprehensive environmental assessments for the DOD.

II. CBR Capacity

A. Introduction to the CBR

The mission of the Center for Bioenvironmental Research (CBR) *is to conduct and coordinate research and teaching to enhance global understanding of environmental issues and provide solutions through innovative communication and technology.*

Founded in 1989, the CBR is an innovative and effective partnership between a Historically Black College or University (HBCU) and a major research university that encourages scientists from multiple disciplines to work together to investigate and resolve environmental problems.

Under the leadership of Dr. John McLachlan, Weatherhead Distinguished Professor in Environmental Studies at Tulane University and an internationally recognized environmental scientist and administrator, the CBR has earned a reputation for its scientific research into the environmental problems of Louisiana. In extending its spheres of influence to national and global problems of the environment, the CBR has brought unique focus that reflects a community-based perspective in conjunction with scientific rigor.

CBR programs are organized around five themes:

Partnerships: The CBR integrates faculty and students from Tulane's Schools of Engineering, Liberal Arts and Sciences, Medicine and Public Health and Tropical Medicine and Xavier's Colleges of Arts and Sciences and Pharmacy in innovative ways to optimize interdisciplinary teaching, learning and research.

Human and Ecosystem Health: Integrating diverse disciplines, the CBR has developed a holistic research program focusing on the effects of environmental hormones on humans and ecosystems through the processes of environmental signaling by natural and synthetic hormones and contaminants that mimic those substances.

Water: The CBR has co-evolved its programs on Environmental Signaling and Aquatic Ecosystem Research to create effective connections. Research efforts employ laboratory and field-scale approaches to look at physiochemical, biological, and ecological impacts in the Mississippi River, Gulf of Mexico, and other aquatic and atmospheric ecosystems.

Communication & Technology: The CBR provides research-based knowledge on the origins, interactions and fate of natural and synthetic chemicals in living systems using informatics capacity particularly its data management and GIS teaching lab. Through its web-based information programs and networked digital technologies, the CBR makes complex issues understandable and provides a forum for scientific discourse.

Environmental Education: The pipeline programs provide interdisciplinary training and research opportunities for undergraduate, graduate and doctoral students and faculty. Internet-based educational programs and outreach initiatives strengthen science education on campus and in the local community and region.

B. The CBR Tulane/Xavier Partnership – a Unique Model

Tulane and Xavier Universities have developed a close working relationship in the past fourteen years, aided by a common vision of academic excellence and the development of high quality educational opportunities for minorities and women. The Tulane/Xavier partnership is a well-established joint venture between majority and minority universities. It is a complementary relationship with respect to environmental restoration and waste management, with the Xavier University focus on education and graduate work, and the Tulane University emphasis and experience in education, research, and technology development and transfer. Optimizing the research capabilities of Tulane and the educational resources at Xavier, the reputation of both institutions enhances the ability of the CBR to solicit resources, recruit staff and researchers, sponsor conferences, and execute successful marketing of its education and research programs.

The relationship between Tulane and Xavier Universities is the foundation of the CBR and serves as a working model for all its collaborations. Since the CBR integrates academic structures, it has the freedom to advance teaching and research by creating flexible working groups to address specific needs and problems. Administrators and researchers team up with government, private, academic, and community individuals and agencies to make use of the best intellectual and technological resources. The partnerships catalyzed by the CBR exist at local, regional, and international levels resulting in community-based solutions to environmental health problems.

1. Tulane Capabilities

Tulane University has established itself as a powerful engine of economic development for New Orleans and Louisiana. Beyond that, in its 170-year commitment to education, Tulane has developed itself as a good citizen that provides the spark of creativity and knowledge that attracts and nurtures intellectual talent while directing its resources to the needs of the community. Tulane is the largest private employer in Orleans Parish and ranks 5th in the State of Louisiana. Tulane's research, healthcare, and educational activities have a total gross economic impact of about \$1.5 billion a year.

Since its inception Tulane has grown into one of the nation's premier institutions of higher learning, known widely for both its undergraduate teaching and cutting-edge research. Those achievements are reflected in rankings by national periodicals such as *US News and World Report* that in 2002 ranked Tulane 43rd overall among all national universities. Tulane University is ranked 14th among private universities in technology transfer and among the top 25 overall in amount of federal research funding.

The University enrolls a diverse student body of 12,381 students from all 50 states and 78 foreign countries in its undergraduate, graduate and professional schools. The University ranks in the top 10 in Environmental Law Studies, the top 15 in Public Health and International Law, and the top 25 in Biomedical Engineering. Tulane is a member of the Association of American Universities and also a Carnegie Research I University.

2. Xavier Capabilities

Xavier University of Louisiana is the nation's only institution of higher education that is historically Black and Catholic. The ultimate purpose of the University is the creation of a more just and humane society. To this end, Xavier prepares its students to assume roles of leadership and service in society. This preparation takes place in a pluralistic teaching and learning environment that incorporates all relevant educational means, including research and community service.

Xavier University has grown in enrollment in the past 10 years with a current enrollment of 3,994 students from 38 states and 27 foreign countries. The student body is predominantly African American (89%), but the university is open to all. More than half of its students major in the natural or health sciences.

Xavier is first nationally in the number of African American students earning undergraduate degrees in the sciences. From 40 to 47% of all recent graduates enroll in professional and graduate schools. The preeminence of Xavier in training undergraduates in science is reflected in its first-place ranking for the past 9 years in the placement of African-American graduates in medical schools. The National Science Foundation has designated Xavier University as one of six to participate in its "Model Institutions for Excellence in Science, Engineering, and Mathematics" program. The Southern Association of Colleges and Schools accredits Xavier University.

C. CBR Support Cores

The state of the art CBR facility at Tulane Health Sciences Center contains sophisticated research equipment including exposure chambers for respiratory disease studies and a Geographical Information Systems (GIS) lab. The facility is electronically networked and physically connected to Tulane Medical School and University Hospital, thus providing easy access between laboratories and clinics. The CBR facility contains state-of-the-art research equipment including a microarray core facility, a fluorescent activated cell sorter, and a high performance gas-liquid chromatograph/dual mass spectrometer. In terms of molecular biologic capabilities, the labs also feature analytical devices, and cellular and molecular biology equipment, tissue-culture facilities, and various containment and decontamination hoods and devices, so that radiation research, protein analysis, PCR, RNA and DNA analyses and immunohistochemical and *in situ* hybridization procedures are routinely conducted in the course of experimentation.

The CBR also has research space on the Tulane University uptown campus. The CBR Uptown contains an additional GIS lab and many equipment cores and lab space for studying neuroscience, molecular biology, and analytical chemistry. The CBR faculty located at Xavier University utilizes modern research and office space in the new seven-module environmental toxicology research center.

1. Research Support

The CBR has a first-rate management team in place with administrative capabilities to develop, implement, facilitate, track, and monitor grants and contracts as well as provide

programmatic direction and administrative leadership for the facility. This team includes a director, a deputy director, associate directors, program managers and coordinators, accountants and administrative secretaries. Tulane and Xavier are innovators among universities in facilitating the mechanisms that allow scientists from multiple disciplines to work together in resolving environmental problems. In addition, the organizational structure of the CBR has allowed it to qualify for large integrated, interdisciplinary grants that are beyond the scope of many other universities or research organizations, including individual Tulane and Xavier departments. The work of the CBR is strengthened by this partnership that can provide the faculty, students, and resources that are necessary to conduct the bioenvironmental hazards research projects discussed in this report.

2. Computer Operations

The CBR has established a Computer Operations Core to modernize and facilitate efficient data communication, sample tracking, QA/QC, and data dissemination. Presentation capabilities are enhanced by a state-of-the-art computer operations facility that provides graphics and electronic media-based services and lab-based microscopy with a digital camera and PC with Image Pro archiving and image analysis software.

The Center provides fast, accurate access to the results generated by high tech analytical instruments located in various Tulane and Xavier scientific laboratories. The SGI equipment is primarily utilized for molecular modeling and other supercomputing tasks that require symmetric multiprocessing and geometry engines for advanced 3-D rendering. Apple and PC notebooks enable the CBR staff to communicate with the department by establishing a link for Internet access, electronic mail, and data transfers while attending events away from the home base. In addition to the equipment currently maintained at satellite locations, the CBR has shared access to Tulane University distributed computing environment of high performance RISC computers. These machines enable the Tulane community to stay connected with other institutions that are part of Internet 2 at data transfer speeds in excess of 100 Mbit/sec as well as other Internet entities at 10Mbit/sec.

During the ONR project period, the Computer Operations Core established and maintained the IT infrastructure necessary to accommodate all project requirements for analysis and information exchange. Collaborative efforts were completed in the establishment of multi-platform wireless connectivity on and off-campus. As part of the effort to maintain these services the Computer Operations Core acquired various hardware and software, implemented wireless networking protocols, managed web-based information exchange, and instituted large monitors for demonstration of research concepts and web editing tools. The Core researched the effectiveness of remote conferencing among researchers for virtual meetings.

3. Environmental Informatics

During the ONR project period, the Environmental Informatics Core provided spatial (GIS) analysis, remote sensing, mapping and other cartographic product development in collaborative projects with BHRP researchers. The Core validated the underlying causes

behind the patterns of bathymetric change discovered and measured in previous years of ONR funding.

4. Communication and Education

Funds were allocated from the Research Support core to cover student support for research teams. In this way the CBR can assist research faculty to build and train sufficient qualified personnel to complete research and also create capacity in undergraduate students. Funds were also provided for two projects in the Communication and Education core for training of students through educational initiatives. They were: 1) one education pipeline pilot program; and 2) the annual international symposium of presentations, posters and workshops on environmental signaling (the *e.hormone* conference).

Education Pipeline Pilot Program: With ONR support, the CBR continued the successful SPRITE program as part of its undergraduate education pipeline initiative to increase the number of African American students enrolling in graduate science programs. The Summer Pipeline Research Initiative: the Tulane Experience (SPRITE) provided Xavier undergraduate students a graduate-level laboratory research experience and mentored introduction to graduate life. In summer 2003, six students were competitively selected from a pool of 20 applicants for the 10-week summer program.

The summer 2003 program involved faculty research mentors from Tulane University Departments of Environmental Health Sciences, Microbiology/Immunology, Ophthalmology, Pathology, Pharmaceutical Sciences, and Pharmacology. In spring 2004, two of the six competitively selected students were accepted to post-baccalaureate schools programs. Of the two, one was accepted to Louisiana State University Medical School, and the other was accepted into a University of Michigan graduate program. The remaining four students have one more year of undergraduate schooling before submitting such applications. In four years, this program has become a major pipeline of minority students to Tulane graduate and health professional schools.

International Environmental Hormone Symposia: One of the central themes of the CBR's Integrated Bioenvironmental Hazards Research Program is the impact of bioenvironmental contaminants on the health of humans and wildlife and their progeny through disruption of the endocrine system. Understanding the many issues surrounding environmental endocrine disruption, or environmental signaling (eg. contaminants and pollutants) and its effects on human and ecosystem health requires a synthesis of disciplines ranging from molecular biology to systemic population biology. For the past five years, the annual Environmental Hormone Symposium (*e.hormone*) in October initiated and hosted by the CBR has been a national and international focal point for all those who are interested in the field of environmental signaling.

The goal of the *e.hormone* symposium series is to bring together innovative thinkers, cutting edge researchers, and key decision makers to critically evaluate current research on environmental signaling and contribute to the future of this field. The symposium format includes scientific presentations grouped around conceptual themes. Preeminent experts in

the field introduce sessions and provide historical perspective on their topic and highlight recent findings. Presentation topics range from human to ecosystem health and from basic research to population studies and all explorations were at the cutting edge of research and policy. The primary strength of this annual forum of information exchange and collegial interaction is its multidisciplinary and multinational nature. Each of the past four symposia has been reported on the web, and its scholarship recognized in publications such as *Science News* and *the Annals of the New York Academy of Sciences*. *e.hormone 2003* was host to over 150 participants and speakers (21 international).

In summary, the CBR is dedicated to training students for careers in science. The CBR sponsors numerous programs to increase and enhance undergraduate, graduate, and faculty research and training opportunities at Tulane and Xavier Universities. A primary CBR goal is to encourage and enhance minority participation and representation in the sciences. The Communication and Education core reflects a collection of enriching environmental education programs that promote awareness of pertinent issues, offer interactive encounters between young and veteran scientists, and provide career-building research experiences in bioenvironmental, biomedical, and environmental sciences.

III. OVERVIEW OF RESEARCH

A. Introduction

Research associated with environmental problems of importance to DOD has required an integrated approach from fundamental science to communication of research results. This report provides a set of integrated research modules that will continue the CBR/ONR BHRP partnership and serve as a model for other DOD research along environmental lines. CBR has earned a reputation for scientific research into relevant environmental problems, while becoming a model of academic/government/industry interaction. Transcending traditional academic structures, CBR provides a powerful tool for modernization of teaching and research.

These research projects integrate common research themes of environmental signals and ecosystem monitoring as well as common technologies of biosensors or biomarkers. Computer Operations and Environmental Informatics cores ensure integration of this research in conjunction with the Communication and Education core, which elucidates communication of this research and the flow of information to future scientists and engineers. One of the benefits of this modular approach is the applicability of these approaches to environmental problems using research themes or technologies that are common across platforms. An additional benefit of these reports is the provision of a mechanism for the bi-directional flow of initiatives and insights from the Navy to and from academia, while providing for the education of future scientists.

In the area of Environmental Signals and Sensors, six projects were funded. Of these six, three were led by Tulane investigators, and two were led by Xavier investigators. One was a joint Xavier/Tulane effort, led by the Xavier investigator. In the area of Ecosystem

Monitoring and Assessment, one project was funded. In the Communication and Education area, two projects were funded. One was a joint Tulane/Xavier effort. A total of nine (9) research projects were funded under the CBR BHRP award of July 2003.

B. Environmental Signals and Sensors

A principal goal of our ongoing research is to examine the actions of DOD-relevant contaminants/pollutants (e.g., organo-chlorine compounds, PAH's, and heavy metals) on important cell targets. Long-term goals of the research in this module are to identify suitable biological markers that will serve as early indicators of toxicant exposure in humans and wildlife and potentially of the overall health of the ecosystem, thereby linking with the other research module in this project. The research addressing this theme will elucidate a scientific basis to develop rational biologically-based risk assessment models.

The projects related to this theme apply molecular biology to elucidate mechanisms of toxicity of these signals and to develop new methods for sensing of toxicants in the environment. Understanding the mechanisms of these toxic reactions permits the development of real understanding of the hazards posed by various contaminants. The data derived from these projects will yield scientific bases to assess risk to humans and wildlife.

In the area of **Environmental Signals and Sensors**, six (6) projects were funded.

- Dr. Karla Johanning, Senior Research Scientist, with Co-Investigator Dr. John McLachlan, Professor of Pharmacology, Tulane University, is investigating the interaction of and endocrine disruptor, HIF (hypoxia inducible factor), thereby leading to potential developmental defects and/or disease states (*"Assessment of molecular interaction between low oxygen and the estrogen receptor in fish cell culture"*).
- Dr. Barbara Beckman, Professor of Pharmacology, with Co-Investigator Dr. John McLachlan, Professor of Pharmacology, Tulane University, is characterizing the signaling pathways and identifying mechanisms by which selected environmental agents and ceramide analogs can subvert the estrogen and cell survival signaling pathways thereby leading to potential dysregulation of biological functions (*"Effects of xenobiotics and endocrine disrupters on breast epithelial cells"*).
- Dr. John McLachlan, Professor of Pharmacology, Tulane University, is investigating the link between HMGA's (High Mobility Group proteins), estrogen, and leiomyomas (fibroids) so as to understand mechanisms that can disrupt normal cell growth, but not form malignant tumors (*"Molecular characterization of human immortalized uterine myometrial and leiomyoma cell lines with emphasis on HMGA 1 and HMGA 2"*).
- Dr. Shubha Kale Ireland, Associate Professor of Biology, Xavier University, is testing the effects of low-level exposures of certain commonly found environmental pollutants, particularly heavy metals, on the frequencies of L1 retrotransposition (*"L1 retrotransposition: a biomarker for exposure to low-levels of environmental pollutants"*).
- Dr. Tanya McKinney, Assistant Professor of Biology, Xavier University, is studying phenotypically and genotypically gram negative (fecal and non-fecal)

bacteria in the lower Mississippi River that are resistant to a panel of commonly used antibiotics (*"Identification and characterization of antibiotic resistant riverine gram negative bacteria"*).

- Dr. Robert Blake, Professor and Chair of Basic Pharmaceutical Sciences, Xavier University, and Co-Investigator Dr. Diane Blake, Professor of Biochemistry, Tulane University, are developing biosensors that will permit the rapid automated identification and quantification of environmental contaminants (*"Characterization of novel antibodies for autonomous underwater vehicles"*).

C. Ecosystem Monitoring and Assessment

The Navy requires a fundamental understanding of fate, transport and transformation effects of contaminants in estuarine and near-shore environments. Since DOD operations can frequently result in the release of a variety of perturbations in a region, a holistic assessment of potential biohazard impacts must include ecosystem-level analyses. Through basic and applied research, the CBR is developing sensor devices that will monitor potential and actual exposure of troops in the field to harmful chemical or biological agents. CBR research emphasis is on providing innovative cost-effective solutions to prevent, minimize, or remedy human health or ecological hazards. CBR expertise on environmental characterization, monitoring, and assessment achieved provides an essential segue into practical research on appropriate environmental impact assessments and management based on that assessment. Research results will advance the current state of knowledge in remediation policies (including determinations of self-remediation) and can result in substantial cost savings for the DOD and other public and private entities in future environmental impact assessment efforts.

In the area of **Ecosystem Monitoring and Assessment** one (1) project was funded.

- Dr. Douglas Meffert, Eugenia Schwartz Professor for River & Coastal Studies, with Co-Investigator Rich Campanella, Research Assistant Professor of Earth and Environmental Sciences, Tulane University, are addressing sediment supply issues for coastal restoration, specifically the analysis of the historical bathymetry of the 210-mile stretch of the Mississippi River from Baton Rouge to the delta (*"Environmental Implications of Mississippi River Bathymetric Patterns"*).

IV. SUMMARY ACCOMPLISHMENTS

A. Overview

Accomplishments on this grant can be documented in three major areas:

1. **Research Publications, Abstracts and Presentations** – that document progress made in the research through publication in a number of peer-reviewed venues available to the general scientific community;
2. **Development of Useable Technologies** – which have direct benefit on furthering the mission-related scientific interests of ONR; and
3. **Intellectual Development** - that extends the ability of the grantee and ONR by developing the next generation of scientists.

For final investigative research reports, see Appendix A.

Research Publications, Abstracts and Presentations:

Research from this project period resulted in the following publications in scientific journals and conference reports:

- Sixteen (16) publications in research journals and in conference reports such as *Annals of New York Academy of Sciences*, *American Geophysical Union*, *Biochemistry*, *Carcinogenesis*, *Experimental Biology & Medicine*, and *International Journal of Oncology*.
- Six (6) published abstracts in meeting and symposium reports.
- Eleven (11) presentations at major scientific conferences across the country and internationally including, but not limited to, the annual meetings of the Estuarine Research Federation, Society for Environmental Toxicology & Chemistry, Society for Integrative & Comparative Biology, and the Society of Surgical Oncologists.

A complete listing of the publications, abstracts and presentation made by investigators on this project can be found in Appendix B.

Development of Useable Technologies:

Research results from this project are generating two useable technologies.

Two technologies that are part of the Environmental Signals and Sensors area are:

- Immunosensor for AUV Deployment. The immediate objectives of this overall project have been to conduct detailed fundamental studies on the binding properties of three novel antibodies (designated as 1B11, 5B2, and 2D42) that are destined to be incorporated into the Autonomous Underwater Vehicle (AUV) as part of our ongoing developmental activities. The antibody-based biosensor will automatically collect and analyze 5 separate samples after installation in an autonomous underwater vehicle or immobilized buoy. A self-contained, automated immunosensor will have the capability to detect very low concentrations of environmental contaminants and/or chemical and biological weapons in surface waters. An assay that detects nanomolar levels of EDTA, the first analyte to be developed for this instrument, has been established. Transfer of the assay to the immunosensor will begin when Sapidyne has corrected the defects in the optical components of the instrument. Sapidyne Instruments (Boise, ID) is constructing the immunosensor with the Tulane laboratory.
- In vivo Cell Culture Models. These models have been established for the examination of gene expression relevant to genetic and possibly environmental contaminants. The cell systems can be utilized to screen differential gene expression. This type of screening would provide information as to potential deleterious effects of certain genetic and environmental chemicals or methodologies to classify these chemicals based upon gene expression profiles.

Further details on the potential technology products can be found in Appendix C.

Intellectual Development:

The research effort provided for the intellectual development of the faculty who participated in the project. In the process of conducting their research, investigators collaborated with new and existing partners in research, and at times formed unique consortia and research teams. Several of the investigators worked across departmental boundaries and in a few instances, faculty members from each component university formed a Tulane/Xavier research team to undertake a project.

The project has supported the research work of:

- 4 graduate students working with 2 Tulane investigators.
- 8 undergraduate students working with 3 Xavier investigators. These undergraduates conducted research with a variety of investigators and thus were exposed to a variety of aspects of the overall project.
- 6 SPRITE (undergraduate) students working with 6 Tulane investigators. They were chosen out of an applicant pool to follow a mentored graduate-level laboratory research experience and gave a scientific presentation of their research project results to mentors, faculty, and peers at the conclusion of the summer program.

A complete listing of the student names and principal investigators/advisors can be reviewed in Appendix D.

B. Environmental Signals and Sensors

- Real Time PCR (RT-PCR) analyses indicate that estrogen treated cell lines under normal oxygen conditions express the vitellogenin gene.
- Utilizing the same RT-PCR analyses, when cell were subjected to hypoxic conditions, ARNT and HIF gene expression was detected.
- *in vivo* screening technologies for AP-1 activating chemicals were developed using stably transfected human endometrial and human embryonic kidney cell lines.
- Common targets exist for the organochlorine/p38-MAPK cascade in the regulation of environmental responsive gene expression.
- Role characterized for organochlorine pesticides and flavonoid phytochemicals signaling to AP-1 via ER-independent mechanisms
- The progesterone receptor is increased in tumor tissues compared to normal tissues.
- ER- β , not ER- α , was increased in tumor, but not in normal, tissues.
- HMGAs were not found to have any differences between the tumor or normal tissues.
- Reproducible data obtained on the effects of several heavy metals on increased L1 retrotransposition, thus establishing the optimized assay as a reliable biomarker of the genotoxic effects of certain environmental pollutants.
- Preliminary data obtained on the possible mechanisms responsible for the stimulatory effects of certain heavy metals on L1 retrotransposition.

- Major genera of the 302 bacteria identified from river samples were *Aeromonas*, *Chromobacterium*, *Enterobacter*, *Burkholderia*, and *Pseudomonas*. The majority of the isolated organisms were resistant to ampicillin, amoxicillin/clavulanic acid, and erythromycin.
- Despite similar water conditions (temperature and pH), *Burkholderia* strains isolated from two River sites were resistant to all antimicrobials tested except ciprofloxacin
- Bacteria isolated from two other River sites were susceptible to ciprofloxacin, sulfamethoxazole/trimethoprim, and in some cases tetracycline.
- First detailed descriptions of extreme allosteric binding behavior by antibodies produced that provide insights into a fundamental property of antibody functional behavior previously unnoticed.
- Since the occurrence of positive cooperativity in antibody-antigen binding interactions has not been described, the possible impact and role of this behavior on the efficacy of the immune system are unknown.
- Once the prevalence and molecular mechanisms of these unexpected phenomena are better understood, it is anticipated that future investigations can focus on the possible exploitation of this phenomenon to improve the performance characteristics of immunosensors.

C. Ecosystem Monitoring and Assessment

- New single-beam SONAR bathymetry data for the lower Mississippi River were acquired from Army Corps of Engineers, processed and integrated into the bathymetric-change research project.
- Eight historical bathymetric datasets of the lower river, collected between 1893 and 1992, were processed, geo-referenced, and differenced for both average depth and deepest point per river mile in ongoing analysis of whether there is sufficient supply of sediments to be diverted from the river and into the wetlands for coastal restoration.

APPENDIX A.

INVESTIGATOR RESEARCH REPORTS

Environmental Signals and Sensors

Assessment of molecular interaction between low oxygen and estrogen receptor in fish cell culture

Principal Investigator: Karla Johanning, Ph.D.
Research Associate Professor
Center for Bioenvironmental Research
Tulane University

Co-Principal Investigator(s): John A. McLachlan, Ph.D.
Professor and Director
Center for Bioenvironmental Research
Department of Pharmacology
Tulane University

Reporting Period: July 2003 – August 2004

Primary Objectives of Research Activities

The main goal of this project is to investigate the interaction of HIF (hypoxia inducible factor) and the estrogen receptor in fish cell lines. Hypoxia may be considered an endocrine disruptor since reproductive success as a whole at the organismal and population levels may be affected. Most of the research in the field of hypoxic events has been carried out in mammalian systems due to the fact that hypoxia is an important factor involved in angiogenesis, tumorogenesis and cardiac disease. The cellular and molecular mechanisms underlying this interaction in other systems such as in fish are not well elucidated. Aquatic hypoxia is increased in thousands of miles of coastal areas due to many factors. Among these, the most alarming is the anthropogenic activity where pollutants are discharged in the water column decreasing the amount of dissolved oxygen necessary for the aquatic life.

Progress Made to Achieve this Objective

Exposure of fish to low oxygen levels (hypoxia) is associated with retarded gonadal development, reduced spawning success, fertilization success, hatching rate, and larval survival. Based upon these observations, aquatic hypoxia has been considered a disruptor of endocrine function in fish. Here, we test for specific interaction between hypoxia and estrogen in mediating gene expression in cultured fish cells. The cellular responses to hypoxia and estrogen are mediated, in part, by the hypoxia-inducible factor (HIF) and the estrogen receptor (ER), respectively.

HIF is a heterodimeric transcription factor that plays a central role in oxygen-regulated gene expression in mammals. During hypoxia, the HIF- α subunit accumulates, dimerizes with ARNT (aryl hydrocarbon receptor nuclear translocator), and together with accessory proteins forms a transcriptional complex that regulates the expression of a number of genes associated with anaerobic metabolism, erythropoiesis, and angiogenesis. Upon estradiol stimulation, the estrogen receptor is activated, dimerizes and forms a transcriptional complex that binds to specific EREs (estrogen response elements) expressing specific genes such as vitellogenin in the liver of the fish.

Homologs of HIF subunits and estrogen receptors are present in fish. In previous work, we have characterized oxygen-dependent reporter gene expression in two cell lines from rainbow trout, RTG-2 (gonad) and RTH-149 (hepatic). When transiently transfected with a plasmid containing a putative hypoxia response element upstream of the luciferase gene, reporter gene expression was maximal at the lowest oxygen level tested (0.5%) at 48 h. In current experiments, both cell lines are exposed to hypoxia in the presence of an agonist (17- β estradiol) or an antagonist (ICI 182,780) of the estrogen receptor. We will measure levels of mRNA and protein of HIF- α , ARNT, estrogen receptors, and selected down-stream genes (e.g., vitellogenin).

Major Accomplishments

In the RTG-2 (rainbow trout gonads) and RTH-149 (rainbow trout hepatocytes):

- When transiently transfected oxygen-dependent reporter gene expression in two cell lines from rainbow trout, RTG-2 (gonad) and RTH-149 (hepatic) with a plasmid containing a putative hypoxia response element upstream of the luciferase gene, reporter gene expression was maximal at the lowest oxygen level tested (0.5%) at 48 h.
- Real Time PCR (RT-PCR) analyses indicate that estrogen treated cell lines under normal oxygen conditions express the vitellogenin gene.
- Utilizing the same RT-PCR analyses, when cell were subjected to hypoxic conditions, ARNT and HIF gene expression was detected as expected.

Publications, Manuscripts, Abstracts

Johanning, K; Lee, J; Wiese,T; Beckman, B; McLachlan and Rees, B. (Assessment of molecular interaction between low oxygen and the estrogen receptor in fish cell culture. 25th Anniversary SETAC (Society for Environmental Toxicology and Chemistry) Conference. November 15-19. 2004. Portland, OR. Abstract submitted and accepted.

Useable Technologies

During the funding period in vivo cell culture models have been established for the examination of gene and protein expression to determine the effects of hypoxia in normal cellular and molecular processes.

Characterization of Novel Antibodies for Autonomous Underwater Vehicles

Principal Investigator: Robert Blake II, PhD.
Professor and Chair
Division of Basic Pharmaceutical Sciences
Xavier University

Co-Investigators: Diane A. Blake, PhD.
Professor
Department of Biochemistry
Tulane Health Sciences Center

Reporting Period: July 2003 – August 2004

Primary Objectives of Research Project

The long-term objective of this research is to develop biosensors that will permit the rapid automated identification and quantification of environmental contaminants. A set of high affinity, highly selective binding reagents (antibodies) is envisioned that will permit the development of portable immunosensors that can rapidly and accurately quantify environmental antigens of interest in real time in the field. The immediate objectives of the progress summarized herein was to conduct detailed studies on the binding properties of three novel antibodies (designated as 1B11, 5B2, and 2D42) that are destined to be incorporated into an Autonomous Underwater Vehicle (AUV) as part of our ongoing developmental activities funded by the Office of Naval Research. All three antibodies exhibit unexpected binding properties that must be characterized and understood before either protein is incorporated into the AUV. It is anticipated that these binding studies will also provide fundamental information on the mechanism(s) of the unexpected binding synergy observed with these antibodies.

Progress Made to Achieve these Objectives

Extreme binding behavior has been observed with all three antibodies.

Three monoclonal antibodies were identified that bound their antigens with homotropic positive cooperativity that was characterized empirically by Hill coefficients with values far greater than 2.0. The equilibrium binding data shown in Fig. 1 provide representative examples of this unexpected behavior. Figs. 1A, B, and C present data obtained by kinetic exclusion assays for the equilibrium binding of the metal-free forms of EDTA to antibody 1B11, aminobenzyl-DTPA to 5B2, and aminobenzyl-EDTA to 2D42, respectively. Each set of data were fit to the following equation:

$$\text{Fraction of occupied sites} = [X]^n / ([X]^n + (K_{0.5})^n) \quad (1)$$

Where $[X]$ is the concentration of the antigen chelator, $K_{0.5}$ is the concentration of antigen where half of the antibodies' binding sites are occupied, and ' n ' is the Hill coefficient. The parameters for the highly sigmoidal *solid curves* drawn through the data points in each panel were determined from the nonlinear regression fit of the data in each panel to Eq. 1. The values of the Hill coefficients obtained from this fitting exercise ranged from 3.7 to 6.5. For comparison purposes, the *dashed curves* drawn in each panel were generated using a simple one-site homogeneous binding model that imposed equal and

independent binding at the two antigen binding sites. The deviation from hyperbolic behavior apparent in the data shown in Fig. 1 is striking.

Fig. 2A shows the sigmoidal binding curve obtained when purified 2D42 was incubated with different concentrations of metal-free EDTA and assayed on the KinExA. The best fit of the data in Fig. 12A to Eq. 2 was obtained with a Hill coefficient of 3.1. This value, though considerably lower than that of 5.1 required to fit the binding obtained with 2D42 and aminobenzyl-EDTA in Fig. 1C, was nonetheless well beyond the maximum value of 2.0 that one would anticipate for the highly cooperative binding of an antigen to a bivalent antibody. Fig. 2B shows the normal hyperbolic binding curve obtained when 2D42 was incubated with different concentrations of DTPA, which differs structurally from EDTA by only one acetic acid and one ethylene amine group. DTPA bound to the antibody with lower affinity than did EDTA and showed no evidence of homotropic positive cooperativity. Fig. 2C shows a comparison of the binding of EDTA and DTPA to 2D42 using a standard ELISA approach. The concentration of DTPA required to competitively inhibit between 10 and 90% of the binding of soluble 2D42 to the antigen immobilized within the ELISA plates covered a span of approximately 1.8 log units, the normal range anticipated for a binding reaction that conforms to the one-site homogeneous binding model. Conversely, the concentrations of EDTA required to competitively inhibit binding of 2D42 to the same immobilized antigen covered a much smaller span of less than 1.0 log units, an observation consistent with the highly cooperative binding behavior observed using the KinExA. Thus, the results obtained by ELISA were qualitatively identical to those obtained using the KinExA.

Given the remarkably high Hill coefficients required to fit the data in Fig. 1 to Eq. 1, it was of interest to examine the equilibrium binding properties of the univalent Fab fragments derived from the proteolytic cleavage of antibodies 5B2 and 2D42. Fig. 3A shows a comparison of the equilibrium binding isoforms obtained with intact 5B2 (*curve a*) and its Fab fragment (*curve b*). Proteolytic cleavage of the intact 5B2 generated a Fab fragment that bound aminobenzyl-DTPA with lower affinity ($K_{0.5}$ of 8.0×10^{-6} M versus 3.2×10^{-7} M) and a lower degree of positive cooperativity (Hill coefficient of 4.5 versus 6.5) than did the intact antibody. Fig. 3B shows a comparison of the equilibrium binding isoforms obtained with intact 2D42 (*curve a*) and its Fab fragment (*curve b*). In this case, proteolytic cleavage of the intact 2D42 generated a Fab fragment that bound metal free EDTA with higher affinity ($K_{0.5}$ of 3.0×10^{-8} M versus 7.9×10^{-8} M) and a slightly higher degree of positive cooperativity (Hill coefficient of 3.3 versus 3.1) than did the intact antibody. Thus the univalent Fab fragments of 5B2 or 2D42 also exhibit extreme homotropic positive cooperativity in the binding of their respective highly charged antigens. Such extreme cooperativity is indicative of multiple ligands binding to the Fab fragments.

The simple illustration presented in Fig. 4 is offered as a structural hypothesis to account for both the complexity and the apparent multiplicity of binding of metal-free polyvalent anions such as EDTA and aminobenzyl-DTPA to antibodies 2D42, 5B2, and their Fab fragments. The Fab fragment of an antibody is depicted by two pairs of two linked ovals, each oval representing a domain in the constant or variable portion of the light or heavy chain, as shown. The traditional antigen-binding site, in this case directed toward a polyvalent anion, is depicted by the short loops extending from the variable portions of the light and heavy chains. A number of potential polyvalent anion binding sites is depicted on the external surface of the Fab that is spatially separated from the antigen-binding site. The location and number of polyvalent anion binding sites shown in the drawing are arbitrary; the illustration is simply meant to convey that many polyvalent anion-binding sites are possible on the Fab. Charged organic anions bound to the external sites are hypothesized to interact with each other and the antigen-

binding site through the apolar core of the proteins to create the complex binding characteristics reported herein.

Figure 1

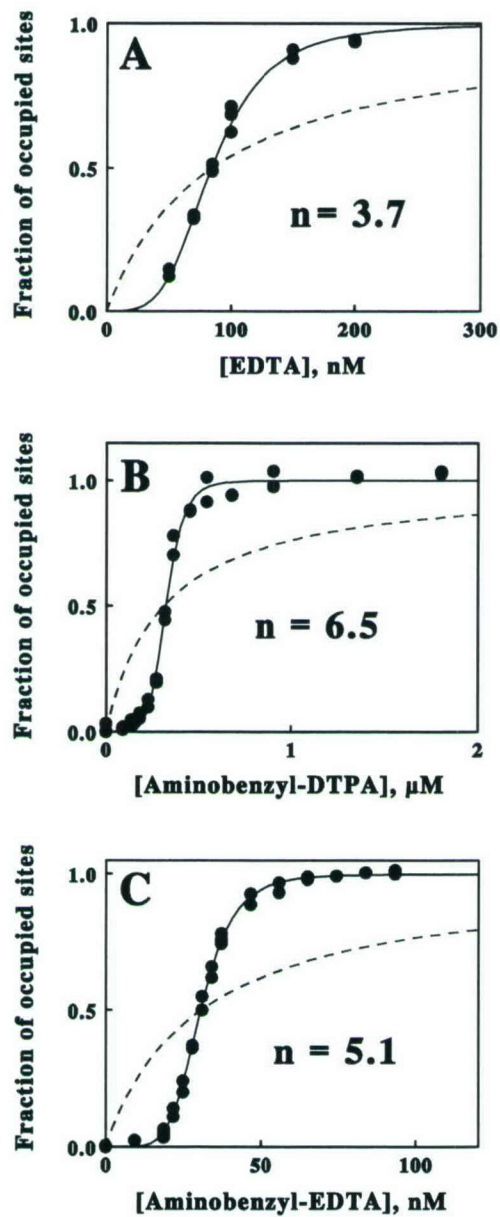


Figure 2

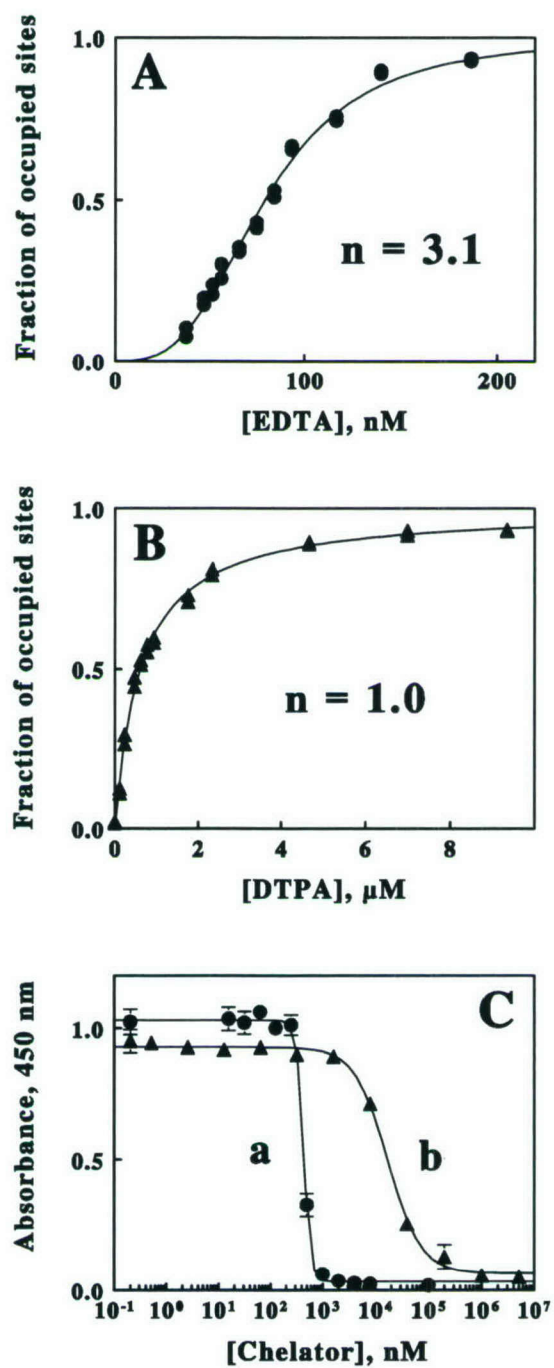


Figure 3

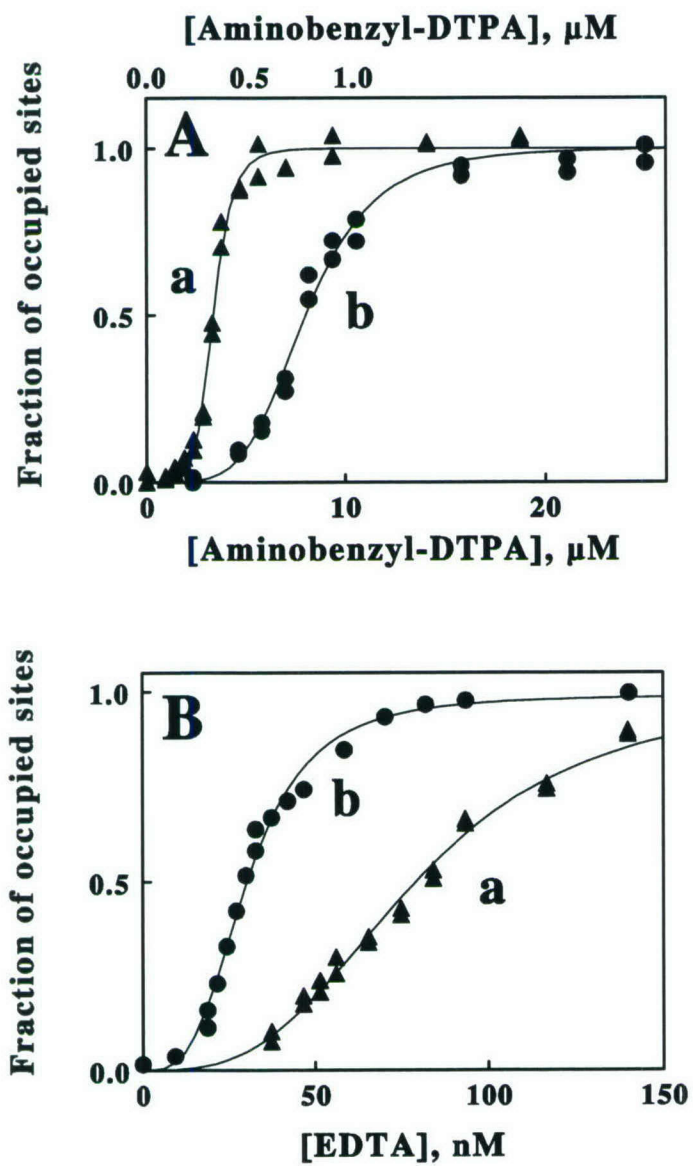
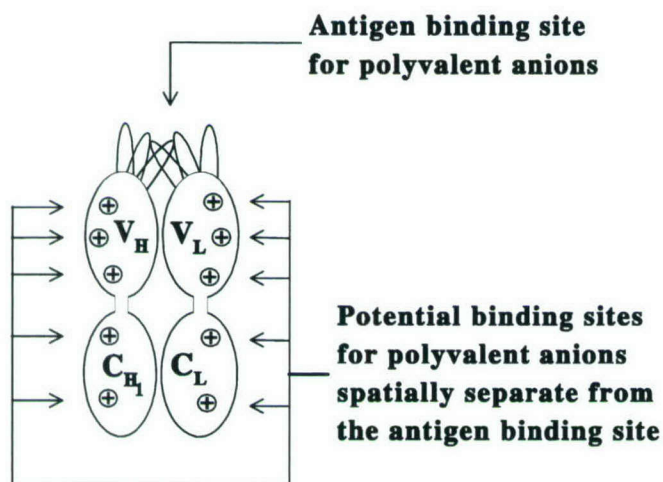


Figure 4



Major Accomplishments

- These are the first detailed descriptions of extreme allosteric binding behavior by antibodies. The observations summarized in this report provide new insights into a fundamental property of antibody functional behavior that appears to have been largely unnoticed. It is anticipated that further studies will contribute to a basic understanding of a heretofore-unrecognized aspect of antibody function. Since the occurrence of positive cooperativity in antibody-antigen binding interactions has not been described, the possible impact and role of this behavior on the efficacy of the immune system are unknown. Once the prevalence and molecular mechanisms of these unexpected phenomena are better understood, it is anticipated that future investigations can focus on the possible exploitation of this phenomenon to improve the performance characteristics of immunosensors.

Publications

Blake, II, R.C., Ohmura, N., Lackie, S.J., Delehanty, J.B., Darwish, I.A., and Blake, D.A. (2003) "Monoclonal Antibodies That Exhibit Allosteric Binding Behavior", accepted for publication in (Columbus, F., ed.) *Progress in Monoclonal Antibody Research*, Nova Science Publishers, Inc., Hauppauge, NY

Presentations

Blake, II, R.C. (2003) "Synergy in antibody-antigen binding interactions", invited seminar at the University of Alabama, Tuscaloosa, AL, October 18

Blake, II, R.C., and Blake, D.A. (2004) "Antibodies that exhibit allosteric binding", DoE-NABIR PI Workshop, Warrenton, VA, March 20

Blake, II, R.C. (2004) "Allosteric binding in antibodies and protein antigens", annual ARCH Research Symposium, New Orleans, LA, May 3

Intellectual Development

1. **Students Trained:** Ms. Dreama Goldsmith, Mr. Wayne Borders, Ms. Tiffany Bolden, and Ms. Teresa Jackson
2. **Period of funding:** Ms. Dreama Goldsmith, for spring, 2004; Mr. Wayne Borders, for summer, 2003; Ms. Tiffany Bolden, for fall and spring; and Ms. Teresa Jackson, for fall and spring.
3. **Brief description of duties and responsibilities:** Each student participated in the instrumental analysis of the equilibrium binding studies using the KinExA flow fluorimeter.

Useable Environmental Technologies

As stated above in Section A, the immediate objectives of the Xavier portion of this overall project were to conduct detailed fundamental studies on the binding properties of three novel antibodies (designated as 1B11, 5B2, and 2D42) that are destined to be incorporated into the Autonomous Underwater Vehicle (AUV) as part of our ongoing developmental activities. The approximate division of work is that the fundamental kinetic and thermodynamic studies are to be conducted at Xavier, while Dr. Diane Blake at Tulane conducts the more applied developmental studies.

L1 Retrotransposition: A Biomarker for Exposure to Low-levels of Environmental Pollutants

Principal Investigator: Shubha Kale Ireland, Ph.D.
Associate Professor
Department of Biology
Xavier University of Louisiana

Reporting Period: July 2003 – August 2004

Primary Objectives of the Research Project

To develop and optimize accurate cell culture assays to measure L1 retrotransposition in mammalian (mouse and/or humans cells).

To test the effects of low-level exposures of certain commonly found environmental pollutants (example heavy metals) on the frequencies of L1 retrotranspositions.

To establish increased L1 retrotransposition as an effective biomarker of exposure to genotoxic chemicals in nature.

To explore the mechanisms by which the heavy metals increase L1 retrotransposition.

Progress Made to Achieve these Objectives

Alu and L1 are among the most active retrotransposons (mobile DNA elements) in the human genome. Several human diseases, including certain forms of breast cancer and leukemia, are associated with L1 and Alu insertions in functionally important areas of the genome. While deleterious consequences of retrotransposition have been documented, little is known about the factors that trigger retrotransposition. Because many environmental pollutants are also suspected carcinogens and heavy metals are ubiquitously found, we have focused our attention on Cadmium sulfide (CdS) the carcinogenic form of this chemical. Using a genetically marked L1 vector and an appropriate control plasmid, which separates the event of retrotransposition from that of cell toxicity, transient assays were conducted using HeLa (cervical cancer) cells. Reproducible results demonstrated a 2.5 to 3.0 fold dose-dependent increase in L1 retrotransposition compared to control cells. The CdS doses tested ranged from 0.38 ppb to 46 ppb and the maximum increase was obtained at 3.8 ppb. To determine whether the L1-stimulatory effects of CdS were associated with increased breaks in the DNA (since several heavy metals are known DNA-nicking agents), Comet assays were performed on CdS-treated HeLa cells using appropriate positive and negative controls. Preliminary results revealed no differences between the negative controls and the CdS-treated cells with respect to 'tailing', a characteristic end-point of the Comet assay. These results suggest that increased L1 insertion is not likely due to increased breaks in the DNA.

Finally, to determine if acetate salts of either Magnesium or Zinc could suppress CdS activity, 9.2 ppb [42.9 nM for $\text{Mg}(\text{Ac})_2$ and 42.0 nM for $\text{Zn}(\text{Ac})_2$] and 92 ppb of each salt were added to 3.8 ppb CdS. In the absence of CdS, neither $\text{Mg}(\text{Ac})_2$ nor $\text{Zn}(\text{Ac})_2$ had any effect on L1 retrotransposition or cell viability. However, both doses of both salts completely suppressed the L1-stimulatory effects of CdS.

These results suggest that the mechanism of action is through displacement of Mg and Zn from cellular proteins, possibly those associated with DNA repair.

Major Accomplishments

- Obtained reproducible data on the effects of several heavy metals on increased L1 retrotransposition, thus establishing the optimized assay as a reliable biomarker of the genotoxic effects of certain environmental pollutants. This work was done in collaboration with Dr. Prescott Deininger (CBR) of Tulane University.
- Obtained preliminary data on the possible mechanisms responsible for the stimulatory effects of certain heavy metals on L1 retrotransposition.

Publications, Manuscripts, Abstracts and Presentations

Abstracts:

Harris, K., Nguyen, T. Q., Nguyen, Q., Brumfield, K., Kwang, S. and **Ireland, S. K.** Comparison of Genotoxic Effects of Particulate Versus Filtered Cadmium sulfide on Human Retrotransposition, Abstract # 1027. Collaborative Workshop in Biomedical Research among Research Centers in Minority Institutions 2003 Spring Symposium (April 28 –29, 2003). Funded by the NIH (National Institutes of Health)

Presentations

Kale¹, S. P., Carmichael¹, M.C., Ross¹, J., Miller¹, J., Sloan¹, C. and Deininger² P., Stimulatory Effects of Particulate CdS on L1 Retrotransposition in HeLa Cells and Possible Mechanisms of Action, ¹Xavier University of Louisiana, New Orleans, LA and ²Tulane University, New Orleans, LA (this was an oral, power-point presentation).

Intellectual Development

1. **Students Trained:** Javay Ross, Rodney Yapi, Jenais Miller
2. **Period of Funding:** 2003 - 2004
3. **Brief Description of Duties and Responsibilities:** All students participated in every aspect of the research, after going through a rigorous training session in safety. They assisted in ordering supplies, conducting experiments and analyzing the results. Each one had to also make a presentation at one of the bi-weekly lab meetings.

Useable Environmental Technologies: None

Identification and Characterization of Antibiotic Resistant Riverine Gram Negative Bacteria

Principal Investigator: Tanya K. McKinney, Ph.D.
Assistant Professor
Department of Biology
Xavier University and
Department of Environmental Health Science
Tulane University School of Public Health

Reporting Period: July 2003 – August 2004

Primary Objectives of Research Project

The primary objectives of this study are (1) to isolate and identify antibiotic resistant gram negative bacteria from various sites along the Mississippi River; (2) to phenotypically characterize isolates through the use of biochemical and enzymatic assays; (3) to assess antibiotic resistance pattern of isolates; (4) to genotypically characterize such isolates through the use of pulse field gel electrophoresis.

Progress made to Achieve These Objectives

Four sample sites located within the city of New Orleans, LA were randomly selected. Site 1 is located downstream of a water treatment plant upstream from the city zoo. Site 2 is located at a ferry landing on the east bank of the river. Site 3 is located in area with a high degree of tourist traffic. Site 4 is located at the ferry site located on the west bank of the river. To date, approximately 403-gram negative isolates have been isolated from Site 1, 215 from Site 2, 289 from Site 3, and 380 from Site 4. Of these only 302 have been identified. Some strains were unculturable following initial isolation. Biochemical analysis using the BBL Crystal Identification system and indole and oxidase testing indicated that the major genera of these bacteria were *Aeromonas*, *Chromobacterium*, *Enterobacter*, *Burkholderia*, and *Pseudomonas*. The majority of the isolated organisms were resistance to ampicillin, amoxicillin/clavulanic acid, and erythromycin.

Antibiogram analysis and comparison was conducted using *Aeromonas hydrophila*, *Chromobacterium violaceum*, and *Burkholderia cepacia* strains isolated from different sites and at different times of year. Spatial and seasonal conditions had no significant effect on the antibiotic resistance patterns of *Aeromonas hydrophila* and *Chromobacterium violaceum* strains isolated from Sites 1, 2, 3, and 4. However, variations were observed for *Burkholderia cepacia*. During the summer of 2003, approximately 49 strains were isolated and identified from the various sites. Despite similar water conditions (temperature and pH), *Burkholderia* strains isolated from site 1 and 2 were resistant to all antimicrobials tested except ciprofloxacin whereas those isolated from site 2 and 4 were susceptible to ciprofloxacin, sulfamethoxazole/trimethoprim, and in some cases tetracycline. No *Burkholderia cepacia* strains, to date, have been isolated and identified in water samples collected during the spring and summer of 2004.

Presentations

Webb, C. and Njoku, V., **Isolation and Identification of Gram Negative Riverine Bacteria.**
Undergraduate Science and Engineering Research Conference, Tuskegee University, November, 2003.

Intellectual Development

1. **Student(s) Name:** Ms. Candice Williams
2. **Period of Funding:** September 2003 - April 2004
3. **Brief Description of duties and responsibilities:** Ms. Williams was responsible for obtaining and processing samples from each site. She plated, isolated, gram stained, characterized, and identified bacteria species.

Useable Environmental Technologies: Not Applicable

Effects of Xenobiotics and Endocrine disrupters on Breast Epithelial Cells

Principal Investigator: Barbara S. Beckman, Ph.D.
Professor
Center for Bioenvironmental Research
Department of Pharmacology
Tulane University

Co-Investigator: John A. McLachlan, Ph.D.
Professor and Director
Center for Bioenvironmental Research
Department of Pharmacology
Tulane University

Reporting Period: July 2003 – August 2004

Primary Objectives of Research Activities

The major goals of this project were to 1) further characterize the signaling pathways triggered in response to relevant organochlorine chemicals, dietary flavonoids, and ceramide analogs that exert effects on estrogen responsive tissues and cell survival pathways, and 2) identify mechanisms by which selected environmental agents and ceramide analogs can subvert the estrogen and cell survival signaling pathways thereby leading to potential dysregulation of biological functions.

Progress Made to Achieve these Objectives

During the funded period we have identified a role for specific signaling pathways including the mitogen-activated protein kinase pathway (MAPK) functioning through AP-1 mediated transcription as a critical component of the estrogen mediated cell survival signaling pathway. The ability of estrogenic chemicals (estradiol, DES, DDT) to exert effects on cell survival pathways of breast carcinoma cells required an intact ERK-MAPK-pathway (1,3). The understanding of the basic mechanisms of cell survival signaling through ER, AP-1 and MAPKs allowed us to develop in vivo screening technologies for AP-1 activating chemicals using stably transfected human endometrial and human embryonic kidney cell lines. These cell systems are continuing to allow us to examine the ability of selected chemicals to activate AP-1 and related signaling pathway (Fos, Jun, Creb, Elk, Chop) through ER-dependent and independent mechanisms (5,6). Subsequent studies have now established that specific organochlorines can activate a number of other signaling pathways including antioxidant response elements, estrogen response elements (ERE), hypoxia-induced factor (HIF) and cyclicAMP response element (CRE). We are, in particular, focusing on the HIF signaling pathway since stress due to hypoxia is an important environmental concern. Our findings thus far suggest that common targets exist for the organochlorine/p38-MAPK cascade in the regulation of environmental responsive gene expression. We are now focusing on the specific roles of MEK5 and HIF in cell signaling events related to estrogenic chemicals and ceramide analogs.

Major Accomplishments

- Identified novel ceramide analogs that can reverse chemoresistance in breast cancer cells
- Identified a role for JNK and p38 MAPKs in signaling by flavonoid phytochemicals in the regulation of ER-mediated gene expression and proliferation of breast carcinoma cells
- Further developed an *in vivo* mammalian cell culture assay to examine environmental relevant organochlorine molecules for estrogen receptor dependent and ER-independent activity toward cell signaling via mitogen-activated protein kinase (MAPK)-mediated activation protein 1 (AP-1) transcription
- Further characterized a role for organochlorine pesticides and flavonoid phytochemicals signaling to AP-1 via ER-independent mechanisms

Publications, Manuscripts, Abstracts

Frigo DE, Vigh KA, Struckhoff AP, Elliott S, **Beckman BS**, Burow ME, **McLachlan JA**. Xenobiotic-induced TNF- α expression and apoptosis through the p38 MAPK signaling pathway. *Carcinogenesis* 25:249-261, 2003.

Simstein R, Burow ME, Parker A, Weldon CB, **Beckman BS**. Apoptosis, chemoresistance and breast cancer: insights from the MCF-7 cell model system. *Exp Biol Med* 228:995-1003, 2003

Weldon CB, Parker AP, Patten D, Elliott S, Tang Y, Frigo DE, Dugan CM, Coakley EL, Butler NN, Clayton JL, Alam J, Curiel TJ, **Beckman BS**, Jaffe BM, Burow ME. Sensitization of apoptotically-resistant breast carcinoma cells to TNF and TRAIL by inhibition of P38 mitogen-activated protein kinase signaling. *Int J Oncol* 24: 1473-1480, 2004

Struckhoff AP, Bittman R, Burow ME, Clejan S, Elliott S, Hammond T, Tang Y, **Beckman BS**. Novel ceramide analogs as potential chemotherapeutic agents in breast cancer. *J Pharmacol Exp Ther* 309:523-532, 2004

Frigo DE, Vigh KA, Struckhoff AP, Elliott S, **Beckman BS**, Burow ME, **McLachlan JA**. Xenobiotic-induced TNF- α expression and apoptosis through the p38 MAPK signaling pathway, *Toxicol Sciences*, submitted, 2004

Burow ME, Colins-Burow BM, Frigo DE, Elliott S, Weldon CB, Boue SM, **Beckman BS**, Curiel TJ, Alam J, **McLachlan JA**. Antiestrogenic activity of flavonoid phytochemicals mediated via the c-Jun N-terminal protein kinase pathway. Cell-type specific regulation of estrogen receptor alpha. *J Steroid Biochem and Mol Biol*, submitted, 2004

Tang Y, Zhao DY, Elliott S, ZhaoW, Curiel TJ, **Beckman BS**, Burow ME. Epigallocatechin gallate (EGCG) induces growth inhibition and apoptosis in human breast cancer cells through inhibition of surviving expression, *Nutrition and Cancer Research*, submitted, 2004

Burow ME, McKee A, Ramsey N, Collins-Burow BM, Melnik LJ, **McLachlan JA**, **Beckman BS**. Inhibition of phorbol ester mediated suppression of TNF-induced apoptosis through blockade of PKC α -MEK-AP1 signaling: A possible mechanism for the anti-tumor effects of apigenin. *Int J Oncol*, submitted, 2004

Burow ME, Duong BN, Frigo DE, Elliot S, Weldon CB, Collins-Burow BM, Alam J, **Beckman BS**, **McLachlan JA**. Crosstalk between PI3K-AKT and the estrogen receptor: a key permissive role in cell survival. *Mol Cell Endo*, submitted, 2004

Burow ME, McKee A, Collins-Burow BM, Frigo DE, Melnik LI, Gozal E, Mallia C, Anderson WB, Alam J, **McLachlan JA**, **Beckman BS**. Potentiation of AP-1 mediated transactivation through inhibition of phosphatidylinositol 3-kinase: involvement of the Erk, JNK and p38 pathways. (in preparation), 2004

Burow ME, Melnik LI, Collins-Burow BM, Tang Y, Elliott S, Alam J, Hill SM, **Beckman BS**, **McLachlan JA**. $G\alpha_{i2}$ - and $G\alpha_o$ -mediated regulation of estrogen receptors (α/β) via coactivator recruitment and activation. (in preparation), 2004

Abstracts

Scandurro AB, Gutierrez YI, Tang Y, **Beckman BS**, Sullivan DE, Morris CA. Integrin-linked kinase: a novel hypoxia-regulated survival factor in human hepatocellular carcinoma cells. *Proc Am Assoc Cancer Res* 44:314, 2003

Figuerola YG, Tang Y, Scandurro A, **Beckman BS**. AKT regulation of MAPK signaling pathway provides a potential cross-talk mechanism for HIF-1 gene-expression in Hep3B cells. *Proc Am Assoc Cancer Res* 44:460, 2003

Curiel TJ, Weldon CB, Parker AP, Patten D, Elliott S, Coakley E, Tang Y, Frigo DE, Butler N, Clayton JL, Alam J, Jaffe BM, **Beckman BS**, Burow ME. P38 MAPK activation blocks breast cancer apoptosis through NF- κ B activation: implications for novel breast cancer treatment strategies with agents inhibiting p38 MAPK activation. *Proc Am Assoc Cancer Res* 44:918, 2003

Tang Y, Weldon CB, Elliott S, Butler NN, Alam J, **Beckman BS**, Curiel TJ, Burow ME. Effects of the MEK5-Erk5 signaling pathway on regulation of cell survival in breast carcinoma cells. *Proc Am Assoc Cancer Res* 44:1363, 2003

Weldon CB, Parker AP, Patten D, Elliott S, Tang Y, Frigo DE, Butler, NN, Clayton JL, Alam J, Curiel, TJ, **Beckman BS**, Jaffe BM, Burow ME. Sensitization of apoptotically resistant breast carcinoma cells to TNF and TRAIL by inhibition of P38 mitogen-activated protein kinase signaling. Society of Surgical Oncologists 56th Annual Cancer Symposia, March 5-9, 2003

Rees BB, Gutierrez YI, **Beckman BS**, Schulte PM. Definition of a hypoxia responsive element in the *Fundulus heteroclitus* *Ldh-B* promoter. Society for Integrative and Comparative Biology, Toronto, CA, Jan. 4-8, 2003

Struckhoff AP, Bittman R, Burow ME, Clejan S, Elliot S, Hammond T, Tang Y, **Beckman BS**. Novel ceramide analogs as potential chemotherapeutic agents in breast cancer. *Proc of the AACR* 45:3746, 2004

Intellectual Development

1. **Students Name:** Yanira Gutierrez-Figueroa, Amanda Parker Struckhoff and Christopher Bertero Weldon
2. **Period of Funding:** YF, 1999-2004; APS, 1999-2004; and CB, 1998-2004
3. **Brief description of duties:**
Yanira Gutierrez-Figueroa (Graduate Student 1999-2004)
(Ph.D. completed July, 2004)
Dissertation title: "The Role of Protein Kinases in Hypoxia-Inducible Factor 1 α -Induced Erythropoietin Gene Expression in Hepatocellular Carcinoma (Hep 3B) Cells"
Present: Post-doctoral fellow, Tulane University

Amanda Parker Struckhoff (Graduate Student 1999-2004)
(Ph.D. completed June, 2004)
Dissertation title: "Mechanisms of the Anti-Proliferative effects of Exogenous Ceramide in Chemoresistant and Chemosensitive Human Breast Carcinoma Cells"
Present: Post-doctoral fellow, L.S.U., New Orleans

Christopher Bertero Weldon (Graduate Student 1998-2004)
(Ph.D. completed May, 2004)
Dissertation title: "Identification and Analysis of the Molecular Mechanisms of Chemoresistance in Human Breast Carcinoma Cells"
Present: Pediatric surgical fellow, Boston Children's Hospital, Boston

Useable Technologies:

It is anticipated that the novel ceramide analogs that we have synthesized and tested in vitro will become potential lead compounds useful in treating chemoresistance in breast cancer.

Molecular Characterization of Human Immortalized Uterine Myometrial and Leiomyoma Cell Lines with Emphasis on HMGA 1 and HMGA 2

Principal Investigator: John A. McLachlan, Ph.D.
Professor and Director
Center for Bioenvironmental Research
Department of Pharmacology
Tulane University

Reporting Period: July 2003 – August 2004

Primary Objectives of Research Activities

The major goal of this project was to investigate the link between HMGAs (High Mobility Group proteins), estrogen, and leiomyomas (fibroids) that will allow us to better understand mechanisms that underlie leiomyomas ability to disrupt normal cell growth but yet restrain from becoming a malignant tumor.

Progress Made to Achieve this Objective

Uterine Leiomyomas are the most common uterine benign tumor occurring in as many as 30% of all women. These tumors are frequent cause of menorrhagia, dysmenorrhea, pelvic discomfort, infertility, recurrent pregnancy loss and hysterectomies. It has been established that uterine leiomyomas growth is dependent on steroid hormones with estrogen being the major factor responsible for leiomyoma development. Previous studies in our laboratory have shown that in leiomyoma tissues compared to myometrial tissues genes Wnt 7a, and DNA Methyltransferase 1 were found to be increased while ER α and DNA Methyltransferase 3A and 3B were decreased. Our current project probes further into the environmental and genetic attributes of these fibroids by using a newly developed leiomyoma cell line. This cell line was genetically altered by human telomerase, which allows the immortalization of the cell line.

During the period funded we have been using real time PCR and probing using Syber green fluorescent dye to be able to detect both HMGAs in cell and tissue samples at the mRNA level. We preformed this experiment to simply test whether or not HMGAs are found in normal myometrial cells and tissues. It has been reported and widely accepted in the literature that HMGAs were not present in normal tissues but found only in tumorigenic tissues. We suspect that by using real time PCR that is a very sensitive technique, we were able to show that indeed they are present in each tissue. Albeit, HMGAs (both A1 and A2) are found increased in leiomyoma compared to myometrial cell lines. Also, since we wanted to establish a link between HMGAs, estrogen, and leiomyomas, we treated cells with four treatments. These were control, estrogen, estrogen + IGF-1 and IGF-1. We found that estrogen only increased mRNA expression levels of HMGA 2 in myometrial cells compared to all other treatments and in treated leiomyoma cells. This was indeed puzzling since we had found that ER α was more than 400 fold expressed in leiomyoma compared to myometrial whereas ER β was expressed at the same fold induction in both cell types. Given that leiomyomas had significantly more ER α we were sure that estrogen treatment would elicit an increased response. We then felt as though since these cells are relatively new, they are genetically altered that they may not be responsive to estrogen. We then probed

for PR (progesterone receptor) in our system using the same treatments. PR is a target gene for estrogen stimulation. We found that PR in leiomyomas estrogen and estrogen + IGF increased PR fold compared to myometrial. This lead us to conclude that ER alpha in leiomyoma cell line may be over saturated and that treatment of estrogen or even IGF would drive induction of ER alpha. Since PR was induced in leiomyomas with estrogen and estrogen + IGF as we thought ER alpha should. To compare, we also probed uterine leiomyoma tissue pairs from a local hospital for ER alpha, beta, HMGAs, and PR to see if there would be any similarities. We found that the only similarity was that of PR being increased in tumor tissues compared to normal tissues. ER beta, not ER alpha was increased in tumor tissues but not in normal tissues. HMGAs were not found to have any differences between either the tumor and normal tissues.

Major Accomplishments

In the leiomyoma and myometrial cell lines:

- HMGA 2 was induced by estrogen treatment in myometrial cells.
- ER (estrogen receptor) alpha expression is greater in leiomyoma cells than in myometrial cells.
- PR (progesterone receptor) was induced by estrogen and estrogen plus IGF-1 (insulin like growth factor 1) treatment in leiomyoma cells.
- HMGAs were increased in leiomyoma cells compared to myometrial cells

In the tissues (tumor vs. normal) was determined:

- In uterine tissue samples, the genetic response of HMGAs, PR and ERs were different than in uterine cells.
- ER beta was increased in tumor samples.
- PR was increased in tumor samples in association with ER beta.
- HMGAs were not found to be differently expressed between tumor and normal tissues.

Publications, Manuscripts, Abstracts

Martin M, Chiang TC, Swartz C, Dixon D, McLachlan JA. (2004). Molecular Characterization of Human Immortalized Uterine Myometrial and Leiomyoma Cell Lines with Emphasis on HMGA 1 and HMGA 2.

This research was presented in the following conferences:

American Association of Cancer Research Conference, Orlando, Florida. March 27-31, 2004. Poster presented.

Tulane University Research Day, New Orleans, LA. April 28-29, 2004. Poster presented.

Reproductive Biology Gordon Conference, New London, CT. June 6-10, 2004. Poster presented.

Intellectual Development

1. **Student Trained:** Martin, Melvenia. (Graduate Student)
2. **Period of Funding:** 2003 - 2004
3. **Duties/Responsibilities:** Maintenance (carry and pass cell lines, feeding regimes for appropriate growth) of the two cell lines: myometrium (smooth muscle cells) and leiomyoma (fibroids), experimentation with those cell lines and such as exposures to estrogen and IGF (insulin-growth factor) treatments to investigate specific gene expression (HMGAs, Progesterone Receptor, Estrogen receptors alpha and beta) utilizing RT-PCR (real Time polymerase chain reaction). In addition, determine gene expression of the above-mentioned genes from preserved tissues.

Useable Technologies

During the funding period in vivo cell culture models have been established for the examination of gene expression relevant to genetic and possibly environmental contaminants. The cell systems can be utilized to screen differential gene expression. This type of screening would provide information as to potential deleterious effects of certain genetic and environmental chemicals or methodologies to classify these chemicals based upon gene expression profiles.

Environmental Monitoring and Assessment

Environmental implications of the Mississippi River bathymetry patterns

Principal Investigator: Douglas J. Meffert, Ph.D.
Eugenie Schwartz Professor for River & Coastal Studies
Deputy Director, Center for Bioenvironmental Research
at Tulane and Xavier Universities;
Program Director, RiverSphere

Co-Investigators: Richard Campanella, MS
Assistant Director for Environmental Analysis
and Research Professor, Earth and Environmental Sciences
Center for Bioenvironmental Research

Reporting Period: July 2003 – August 2004

Primary Objectives of Research Project

The Environmental Informatics Core (EIC, the Geographic Information Systems/ remote sensing/ cartography arm of the Center for Bioenvironmental Research) proposed to ONR to advance its earlier work to address sediment supply issues for coastal restoration, specifically the analysis of the historical bathymetry of the 210-mile stretch of the Mississippi River from Baton Rouge to the delta. Eight historical bathymetric datasets of the lower river, collected between 1893 and 1992, were processed, geo-referenced, adjusted for differences in vertical datums and stage, corrected for errors, interpolated, and differenced for both average depth and deepest point per river mile. Findings were presented at various professional forums in 2002-2003, and were of particular interest because recent coastal restoration efforts have left open the question of sufficient supply of sediments to be diverted from the river and into the wetlands.

The Environmental Informatics Core proposed for this reporting year to develop further these datasets to address the “supply” side of sediment issues. Additionally, we proposed to support other research cores in GIS, remote sensing, cartography, and the development of graphical products, as needed. This included the development of GIS-based products for the development of the RiverSphere.

Progress Made to Achieve these Objectives

New 2003 single-beam SONAR bathymetry data for the lower Mississippi River were acquired from Army Corps of Engineers, processed and integrated into the bathymetric-change research project. Additionally, patches of high-resolution multibeam SONAR were acquired and are currently being processed for use in a state-funded modeling project, which was won in part based on earlier ONR-funded work. River-mapping work was also conducted in support of Dr. Mead Allison (comparison of multibeam bathymetry data of Mississippi River near New Orleans, 2001-2004), for Ph. D. student Laura Wysocki (areal measurements of Mississippi River plume), and others in the River Communications Core. ONR-funded research resulted in the publication of “Biochemical Implications of Levee Confinement in the Lowermost Mississippi River,” *EOS Transactions, American Geophysical Union*, in November 2003, and “Sustainability, Survivability, and the Paradox of New Orleans,” in the *Annals of the New York Academy of Sciences* in June 2004.

ONR-funded time was also spent on the research and development of numerous cartographic, textual, and graphical products relating to CBR's RiverSphere effort, aimed at creating a river-focused research and education facility on the banks of the Mississippi in New Orleans.

Additionally, GIS research in emerging diseases, proposed during 2002 but ongoing into 2004, was conducted in the reporting year. This included the spatial analysis of distributions of key mosquito species at 31 trap sites throughout the New Orleans metropolitan area. This research led to further funding from NIH won by entomologist Dr. Dawn Wesson, in collaboration with the EIC.

Major Accomplishments

- New 2003 single-beam and multi-beam SONAR bathymetry data acquired and processed
- Technical support provided to affiliated ONR-funded researchers
- Research and publication of two co-authored technical papers
- Development of numerous RiverSphere-related maps and graphics
- Additional emerging diseases research (habitat preferences of key arbovirus mosquito species)

Publications

Galler, J.J., T.S. Bianchi, M.A. Allison, L.A. Wysocki, and **R. Campanella**. "Biochemical Implications of Levee Confinement in the Lowermost Mississippi River," *EOS Transactions, American Geophysical Union*, Vol. 84, Number 44, November 2003, pp. 474-475.

Campanella, R., Daniel Etheridge, and **Douglas J. Meffert**. "Sustainability, Survivability, and the Paradox of New Orleans." Urban Biosphere and Society, *Annals of the New York Academy of Sciences*, Vol. 1023, 2004.

Presentations

Abboud, E.R.; **Blake, D.A.**; **Meffert, D.J.**: Biosensors in Estuaries: A Rapid, Sensitive Immunosensor Replacement for Coliform Measurement. Oral presentation at the Estuarine Research Federation 2003 Conference in Seattle, WA, September 14-18, 2003.

Meffert, D.J. and Rey, G.: Real-time Monitoring of Environmental Toxicants through Autonomous Underwater Vehicles and Biosensor Development. Invited oral presentation at the International Conference on Robotics and Automation 2004 in New Orleans, LA, April 26-May 1, 2004.

Intellectual Development: None

Useable Environmental Technologies: None

Partners (academia, industry, labs/centers, federal agency, etc.)
University of Louisiana at Lafayette; University of New Orleans

Patents: None

Research Cores

Computer Operations and Information Technology

Principal Investigator: John Vassilopoulos, MS
Director
Computer Operations
Center for Bioenvironmental Research
Tulane University

Reporting Period: July 2003 – August 2004

Primary Objectives of Research Activities

To provide end-user technical direction for data warehousing, networking, securing storage for project-related data, and to coordinate with research support efforts for all CBR core projects.

Progress Made to Achieve these Objectives

- Hardware and software acquisitions for security and backup procedures.
- Implementation of wireless networking schemes for remote locations.
- Hardware for CD/DVD publishing.
- Web-based information exchange.

Major Accomplishments

- Established and maintained the IT infrastructure necessary to accommodate all project requirements for analysis and information exchange.
- Researched effectiveness of video conferencing among researchers for multi-point meetings that accommodate sound, video, and data transfer. Several platforms were evaluated.

Publications, Manuscripts, Abstracts: N/A

Presentations: N/A

Intellectual Development: N/A

Useable Technologies: N/A

Education and Communication

SPRITE: The Summer Pipeline Research Initiative: the Tulane Experience A Mentored Introduction to Programs of Study for Graduate Research

Principal Investigator: Douglas Meffert, Ph.D.
Deputy Director
Center for Bioenvironmental Research (CBR)
Tulane University

Co – Investigators: Charles E. Allen, III, MSPH
Education and Outreach Manager

Dana M. Greene-McDowelle, Ph.D.
Assistant Professor, Biology
Xavier University of Louisiana

Reporting Period: July 2003 – August 2004

Primary Objectives of Research Activities

The Summer Pipeline Research Initiative: the Tulane Experience (SPRITE) is an educational initiative of the CBR's Research Academy. Tulane University's Molecular and Cellular Biology (MCB) interdisciplinary graduate program. As in the past, the goal of SPRITE is to increase the number of minorities at the graduate level in the bioenvironmental and biomedical sciences by:

- Providing Xavier undergraduate students with a quality bench research experience in an MCB laboratory under the guidance of an established researcher; and
- Exposing these students to graduate life and the MCB program of Tulane University.

The program focuses on two unique resources: Tulane's successful biomedical graduate program and Xavier University's outstanding pool of science majors. The intent of the program is to provide a mentored introduction to Tulane's excellent programs of graduate study with a successful research experience supported by ONR.

In addition to the research, students also participate in weekly seminars and roundtable discussions led by the SPRITE program staff. These seminars cover areas such as research at the frontiers of science, financial aid and career opportunities. Other sessions include forums on the presentation of a research seminar and training on graphics and presentation skills.

The culminating event for the summer activities is a research symposium in which student interns present their work to the assembled faculty, students, laboratory colleagues and staff. Subsequently, in the following fall, students have access to SPRITE staff for assistance in applying to graduate and professional schools for the appropriate academic year.

Progress Made to Achieve These Objectives

In spring 2003, program coordinators developed application packets and disseminated information to eligible sophomores, juniors, and seniors at Xavier University. Six students were competitively selected

from a pool of 20 applicants for the 10-week summer program. Presentations were made to faculty and students at Tulane and Xavier Universities to recruit both mentors and research interns.

The summer 2003 program involved faculty research mentors from the following disciplines: Environmental Health Sciences, Microbiology/Immunology, Ophthalmology, Pathology, Pharmaceutical Sciences, and Pharmacology.

Activities by the SPRITE coordinators in fall 2003 included assistance with student applications to graduate school and other professional schools.

In spring 2004, one of the six interns was accepted to a post-baccalaureate program. One was accepted to Louisiana State University Medical School and another was accepted into a graduate program at the University of Michigan. And, the remaining four students have one more year of undergraduate schooling before submitting such applications.

Selection of SPRITE interns for the summer 2004 program was equally competitive; 19 applications were received for the 6 positions. Funding for this set of interns was provided in total by 2003-04 ONR funding.

The success of SPRITE provides a national model for pipeline partnerships between research institutions and historically black colleges/universities.

Presentations

Dallas, Torijuan, Clinton, Antoine and Hunter, Christina. Presented at the Annual Biomedical Research Conference for Minority Students, October 15-18, 2003, San Diego, CA. The 2003 interns presented at the CBR's summer research academy symposium, which was held on Friday, August 1, 2003 at the CBR.

By Program Coordinators - A poster was presented at a presentation held at Xavier University during a visit of the National Science Board. The poster highlighted all CBR programs along with SPRITE, which are supported by ONR.

Intellectual Development

1. **Student Name:** Antoine Clinton
2. **Funding Period:** May through August 2003; February through May 2004
3. **Duties and Responsibilities:** Student was responsible for conducting research on the project entitled, *P(HEMA) Polymers with incorporated Mitomycin C: A New Device to Control the Fibroproliferative Response after Glaucoma Surgery*.
Research Advisor: Dr. Diane Blake, Ophthalmology, Tulane University Health Sciences Center (TUHSC)
1. **Student Name:** Torijuan Dallas
2. **Funding Period:** May through August 2003
3. **Duties and Responsibilities:** Student was responsible for conducting research on the project entitled, *The Polymorphisms of CYP2C8 Enzyme and Hypertension*.
4. **Research Advisor:** Dr. Shanker Japa, Pathology, TUHSC

1. **Student Name:** Jennifer Hwang
2. **Funding Period:** May through August 2003
3. **Duties and Responsibilities:** Student was responsible for conducting research on the project entitled, *The Role of Genetic Polymorphisms in the Epoxygenase Pathway of Hypertension*.
4. Research Advisor, Dr. Sanda Clejan, Pathology, TUHSC

1. **Student Name:** Christina Hunter
2. **Funding Period:** May through August 2003
3. **Duties and Responsibilities:** Student was responsible for conducting research on the project entitled, *Immunohistochemistry as an Assay for Invertebrate Transgenesis*.
4. Research Advisor: Dr. Sharon Isern, Tropical Medicine, TUHSC

1. **Student Name:** Ryan Jupiter
2. **Funding Period:** May through August 2003
3. **Duties and Responsibilities:** Student was responsible for conducting research on the project entitled, *The Role of Hydrogen Cyanide Production by Pseudomonas Aeruginosa in Patients with Cystic Fibrosis*.
4. Research Advisor: Dr. Michael Schurr, Microbiology/Immunology, TUHSC

1. **Student Name:** Nicole Lee
2. **Funding Period:** May through August 2003
3. **Duties and Responsibilities:** Student was responsible for conducting research on the project entitled, *Phytoestrogens and their Oestrogenic/ Anti-Oestrogenic Effect on the Female Endocrine System*
4. Research Advisor: Dr. Thomas Wiese, Basic Pharmaceutical Sciences, Xavier University

Useable Technologies: None

Symposium on the Environment & Hormones (e.hormone), 2003

Principal Investigator: John A. McLachlan, Ph.D.
Professor and Director
Center for Bioenvironmental Research
Department of Pharmacology
Tulane University

Reporting Period: July 2003 – August 2004

Primary Objectives of Research Project:

One of the central themes of the CBR's Integrated Bioenvironmental Hazards Research Program is the impact of bioenvironmental contaminants on the health of humans and wildlife and their progeny through disruption of the endocrine system. Understanding the many issues surrounding environmental endocrine disruption, or environmental signaling (e.g. contaminants and pollutants) and effects on human and ecosystem health requires a synthesis of disciplines ranging from molecular biology to systemic population biology. This becomes a daunting task since the scientific terminology and methodology, the meetings attended, and literature read by researchers does not usually overlap. The CBR responded to the need for a scientific forum for information exchange and collegial interaction for scientists involved in environmental signaling research by hosting the first international Symposium on the Environment and Hormones (e.hormone) in October 1999. e.hormone has become an annual event.

The goal of this symposium series is to bring together innovative thinkers, cutting edge researchers, and key decision makers to critically evaluate current research on environmental signaling and contribute to the future of this field. The fourth annual e.hormone symposium took place in New Orleans October 16-18, 2003. It was a multidisciplinary, multinational event. Topics ranged from human to ecosystem health, from basic research to population studies. As always, it was an active meeting with lively discussions of the hottest issues; formal and informal networking opportunities were built into the schedule of symposium activities.

e.hormone 2003 was host to over 150 speakers and participants (21 international). Ecologists, chemists, endocrinologists, toxicologists, zoologists, engineers, philosophers, undergraduate science faculty, high school teachers, policy makers, and media from the United States, Japan, Europe, and Latin America came together to analyze the latest findings on environmental signaling that are the basis of endocrine disruption. Explorations remained at the cutting edge in research and policy.

After attending this continuing education activity, the participant should have been able to:

- Interpret cutting edge research and techniques related to environmental signaling and apply current knowledge to future research or decision-making
- Identify research interests and colleagues in the field of environmental signaling that will foster future collaboration or information exchange
- Understand philosophical approaches, concepts, frameworks, and policy implications related to endocrine disrupting chemicals and environmental signaling

- Comprehend the etiology of endocrine disorders such as breast and uterine disease, early puberty, and abnormalities in sexual development and functioning.

Progress Made to Achieve these Objectives

e.hormone sessions were held at the CBR conference facility in the Health and Environmental Research Building and at the Renaissance Pere Marquette Hotel in downtown New Orleans. Participants felt the organization of the program, with several talks on a topic was a welcome antidote to the usual dizzying fragmentation of subjects at conferences. The organizing committee, cognizant of the latest literature and findings, selected presenters who conduct cutting edge research in a variety of disciplines and represent diversity in race/ethnicity, gender, geography, and senior/junior research status. This was an additional strength of the carefully constructed program; it did not merely feature eminent investigators that many in the audience had already heard numerous times; fresh voices with interesting new work were welcome. In particular, the 2003 program featured three new workshops, which allowed for in-depth exploration of and communication on key topics.

e.hormone 2003 featured exciting presentations and information exchanged in a collegial atmosphere with high quality talks. The theme of signaling across organisms and the susceptibility of numerous systems to the deleterious effects of environmental hormones was an effective organizing principle. The session topics, representation from diverse scientists, and the caliber of the talks resulted in a highly productive and enjoyable meeting for all participants.

As part of its NSF-funded Environmental Signaling Network (ESN), the CBR introduced a workshop preceding the e.hormone conference, Environmental Signaling 101: the Basics of Endocrine Disrupter Research (ES101). This course, taught to approximately 50 people, provided a fundamental understanding of the principles underlying endocrine disruption; the interactive workshop focused on the basics of the science and also strategies and techniques for communicating and teaching this complex information. It was open to anyone who registered for the e.hormone conference. The ESN also hosted and provided mentoring to six geographically diverse student fellows at ES101 and e.hormone 2003.

Examples of ONR-related research topics and themes at e.hormone 2003 include the following presentations: Elwood Linney (Duke University) *Transgenic Fish Approaches to Receptor Pathways*; Steve McDonald (Carollo Engineering) *Macro-engineering of Water Quality in the Age of Drugs and Pharmaceuticals*; Herbert Buxton (USGS Toxics Program) *Occurrence and Implication of Hormones and Pharmaceuticals in America's Streams and Waterways*; Patrick Larkin (EcoArray, LLC) *Genomic Approaches to Biomarkers of Effect in Aquatic Species*; James Witliff (University of Louisville) *Xenoestrogen Detection and Assessment with Biosensors and Gene Expression Profiling*; and Xia Li (Tulane University) *Antibody Based Biosensor for Heavy Metals in the Environment*.

Major Accomplishments

Throughout its five-year history, the e.hormone symposium has resulted in the creation of an extensive global network. Major accomplishments include:

- Diversified participation, and symposium continuity – participants came from 9 countries outside the US; many returning attendees as well as new faces in 2003
- Comprehensive poster session for junior investigators - 45 posters
- Novel approaches to science and communication via three conference workshops:

Workshop I – Developmental Estrogenization Syndrome

Through this provocative and enlightening exercise we sought to create a hypothetical model for developmental induction of adult health and disease. Panelists included epidemiologists, molecular biologists, developmental systems biologists, consumers, clinicians, ecologists, and experimental biologists.

Workshop II – Connections in the Environment: Perspectives from the Arts

This workshop addressed a two-fold issue: (1) is environmental signaling a good metaphor in which to help art, science, and the humanities meet? and (2) what can such convergence create that cannot be generated independently? Panelists presented philosophic, personal, movement, & sculptural perspectives.

Workshop III – The River Runs Through Us

Three high school students presented results from their summer research projects investigating water quality and wetland issues. The workshop focused on what we need to do to increase high school student participation in research projects and the reciprocal beneficial relationship for students and investigators. It also asked if there an overall approach to determine impact of biologically active materials in our waterways.

Maintenance of the "spin-off" e.hormone website as a hub of scientific and media information connecting research colleagues throughout the year

Publications (Manuscripts and Abstracts)

Each of the past four symposia has been reported on the web, and its scholarship recognized in publications such as *Science News*.

Presentations

e.hormone 2003 sessions:

- I A Systems Biology Approach to Environmental Signaling
- II Signaling in Gene Networks
- III Developmental Patterning
- IV Developmental Estrogenization Syndrome Workshop
- V Connections in the Environment: Perspectives from the Arts Workshop
- VI Hormones and Pharmaceuticals in Water
- VII The River Runs Through Us Workshop

Intellectual Development: N/A

Useable Environmental Technologies:

While no technologies have resulted directly from the e.hormone workshop series, the CBR deems that interdisciplinary workshops like this one are critical for the creation of scientific collaborative

approaches that foster biosensor development. Such biosensors harness the power of “environmental signaling,” and lead us to further exploration and development of numerous near real-time monitoring technologies for the ONR, in particular, and the DOD, in general.

APPENDIX B.

PUBLICATIONS, MANUSCRIPTS, ABSTRACTS, PRESENTATIONS

Publications, Presentations, Abstracts

Publications

Blake, II, R.C., Delehanty, J.B., Khosraviani, M., Yu, H., Jones, R.M., and Blake, D.A. "Allosteric Binding Properties of a Monoclonal Antibody and Its Fab Fragment", *Biochemistry* 42, 497-508 (2003)

Blake, II, R.C., Ohmura, N., Lackie, S.J., Delehanty, J.B., Darwish, I.A., and Blake, D.A. "Monoclonal Antibodies That Exhibit Allosteric Binding Behavior", accepted for publication in (Columbus, F., ed.) *Progress in Monoclonal Antibody Research*, Nova Science Publishers, Inc., Hauppauge, NY (2003)

Burow ME, Colins-Burow BM, Frigo DE, Elliott S, Weldon CB, Boue SM, **Beckman BS**, Curiel TJ, Alam J, **McLachlan JA**. "Antiestrogenic activity of flavonoid phytochemicals mediated via the c-Jun N-terminal protein kinase pathway. Cell-type specific regulation of estrogen receptor alpha." *J Steroid Biochem and Mol Biol*, submitted 2004

Burow ME, McKee A, Ramsey N, Collins-Burow BM, Melnik LJ, **McLachlan JA**, **Beckman BS**. "Inhibition of phorbol ester mediated suppression of TNF-induced apoptosis through blockade of PKC α -MEK-AP1 signaling: A possible mechanism for the anti-tumor effects of apigenin." *Int J Oncol*, submitted 2004

Burow ME, Duong BN, Frigo DE, Elliot S, Weldon CB, Collins-Burow BM, Alam J, **Beckman BS**, **McLachlan JA**. "Crosstalk between PI3K-AKT and the estrogen receptor: a key permissive role in cell survival." *Mol Cell Endo*, submitted 2004

Burow ME, McKee A, Collins-Burow BM, Frigo DE, Melnik LI, Gozal E, Mallia C, Anderson WB, Alam J, **McLachlan JA**, **Beckman BS**. "Potentiation of AP-1 mediated transactivation through inhibition of phosphatidylinositol 3-kinase: involvement of the Erk, JNK and p38 pathways." (in preparation 2004).

Burow ME, Melnik LI, Collins-Burow BM, Tang Y, Elliott S, Alam J, Hill SM, **Beckman BS**, **McLachlan JA**. "G α_{i2} - and G α_o -mediated regulation of estrogen receptors (α/β) via coactivator recruitment and activation." (in preparation 2004).

Campanella, R., Etheridge, Daniel, and **Meffert, Douglas J.**. "Sustainability, Survivability, and the Paradox of New Orleans." Urban Biosphere and Society, *Annals of the New York Academy of Sciences*, Vol. 1023, 2004. 289-299

Frigo DE, Vigh KA, Struckhoff AP, Elliott S, Beckman BS, Burow ME, McLachlan JA. "Xenobiotic-induced TNF- α expression and apoptosis through the p38 MAPK signaling pathway." *Carcinogenesis* 25:249-261, 2003.

Frigo DE, Vigh KA, Struckhoff AP, Elliott S, **Beckman BS**, Burow ME, **McLachlan JA**. "Xenobiotic-induced TNF- α expression and apoptosis through the p38 MAPK signaling pathway," *Toxicol Sciences*, (submitted 2004).

Galler, J.J., Bianchi, T.S., Allison, M.A., Wysocki, L.A., and **Campanella, R.** "Biochemical Implications of Levee Confinement in the Lowermost Mississippi River," *EOS Transactions, American Geophysical Union*, Vol. 84, Number 44, November 2003, pp. 474-475.

Martin M, Chiang TC, Swartz C, Dixon D, McLachlan, JA. Molecular Characterization of Human Immortalized Uterine Myometrial and Leiomyoma Cell Lines with Emphasis on HMGA 1 and HMGA 2. American Association Cancer Research (AACR) in March 28-31, 2004

Simstein R, Burow ME, Parker A, Weldon CB, **Beckman BS**. "Apoptosis, chemoresistance and breast cancer: insights from the MCF-7 cell model system." *Exp Biol Med* 228:995-1003, 2003

Struckhoff AP, Bittman R, Burow ME, Clejan S, Elliott S, Hammond T, Tang Y, **Beckman BS**. "Novel ceramide analogs as potential chemotherapeutic agents in breast cancer." *J Pharmacol Exp Ther* 309:523-532, 2004

Tang Y, Zhao DY, Elliott S, ZhaoW, Curiel TJ, **Beckman BS**, Burow ME. "Epigallocatechin gallate (EGCG) induces growth inhibition and apoptosis in human breast cancer cells through inhibition of surviving expression," *Nutrition and Cancer Research*, submitted 2004.

Weldon CB, Parker AP, Patten D, Elliott S, Tang Y, Frigo DE, Dugan CM, Coakley EL, Butler NN, Clayton JL, Alam J, Curiel TJ, **Beckman BS**, Jaffe BM, Burow ME. "Sensitization of apoptotically-resistant breast carcinoma cells to TNF and TRAIL by inhibition of P38 mitogen-activated protein kinase signaling." *Int J Oncol* 24: 1473-1480, 2004

Presentations

Abboud, E.R., **Blake, D.A.**; **Meffert, D.J.**: Biosensors in Estuaries: A Rapid, Sensitive Immunosensor Replacement for Coliform Measurement. Oral presentation at the Estuarine Research Federation 2003 Conference in Seattle, WA, September 14-18, 2003.

Blake, II, R.C. "Synergy in antibody-antigen binding interactions", invited seminar at the University of Alabama, Tuscaloosa, AL, October 18, 2003

Blake, II, R.C., and Blake, D.A. "Antibodies that exhibit allosteric binding", DoE-NABIR PI Workshop, Warrenton, VA, March 20, 2004

Blake, II, R.C. "Allosteric binding in antibodies and protein antigens", annual ARCH Research Symposium, New Orleans, LA, May 3, 2004

Dallas, Torijuan, Clinton, Antoine and Hunter, Christina. Dallas, Torijuan-*The Polymorphisms of CYP2C8 Enzyme and Hypertension*. Clinton, Antoine-*P(HEMA) Polymers with incorporated Mitomycin C: A New Device to Control the Fibroproliferative Response after Glaucoma Surgery*. Hunter, Christina-*Immunohistochemistry as an Assay for Invertebrate Transgenesis*. Annual Biomedical Research Conference for Minority Students, October 15-18, 2003, San Diego, CA.

Johanning, K; Lee, J; Wiese, T; Beckman, B; McLachlan, JA and Rees, B. "Assessment of molecular interaction between low oxygen and the estrogen receptor in fish cell culture." 25th Anniversary SETAC (Society for Environmental Toxicology and Chemistry) Conference. Portland, OR, November 15-19, 2004. (Abstract submitted and accepted).

Kale, S. P., Carmichael, M.C., Ross, J., Miller, J., Sloan, C. and Deininger P., "Stimulatory Effects of Particulate CdS on L1 Retrotransposition in HeLa Cells and Possible Mechanisms of Action." First International Symposium on Recent Advances in Environmental Health Research, Jackson State University, September 19 -22, 2004.

Meffert, D.J. and Rey, G. "Real-time Monitoring of Environmental Toxicants through Autonomous Underwater Vehicles and Biosensor Development." International Conference on Robotics and Automation 2004, New Orleans, LA, April 26-May 1, 2004.

Rees BB, Gutierrez YI, **Beckman BS**, Schulte PM. Definition of a hypoxia responsive element in the *Fundulus heteroclitus* *Ldh-B* promoter. Society for Integrative and Comparative Biology, Toronto, CA, Jan. 4-8, 2003

Webb, C. and Njoku, V., Isolation and Identification of Gram Negative Riverine Bacteria. Undergraduate Science and Engineering Research Conference, Tuskegee University, November, 2003

Weldon CB, Parker AP, Patten D, Elliott S, Tang Y, Frigo DE, Butler, NN, Clayton JL, Alam J, Curiel, TJ, **Beckman BS**, Jaffe BM, Burow ME. Sensitization of apoptotically resistant breast carcinoma cells to TNF and TRAIL by inhibition of P38 mitogen-activated protein kinase signaling. Society of Surgical Oncologists 56th Annual Cancer Symposia, March 5-9, 2003

Abstracts

Curiel TJ, Weldon CB, Parker AP, Patten D, Elliott S, Coakley E, Tang Y, Frigo DE, Butler N, Clayton JL, Alam J, Jaffe BM, **Beckman BS**, Burow ME. P38 MAPK activation blocks breast cancer apoptosis through NF-kB activation: implications for novel breast cancer treatment strategies with agents inhibiting p38 MAPK activation. *Proc Am Assoc Cancer Res* 44:918, 2003

Figuerola YG, Tang Y, Scandurro A, **Beckman BS**. AKT regulation of MAPK signaling pathway provides a potential cross-talk mechanism for HIF-1 gene-expression in Hep3B cells. *Proc Am Assoc Cancer Res* 44:460, 2003

Harris, K., Nguyen, T. Q., Nguyen, Q., Brumfield, K., Kwang, S. and **Ireland, S. K.** Comparison of Genotoxic Effects of Particulate Versus Filtered Cadmium sulfide on Human Retrotransposition, Abstract # 1027. Collaborative Workshop in Biomedical Research among Research Centers in Minority Institutions 2003 Spring Symposium (April 28 –29, 2003)

Scandurro AB, Gutierrez YI, Tang Y, **Beckman BS**, Sullivan DE, Morris CA. Integrin-linked kinase: a novel hypoxia-regulated survival factor in human hepatocellular carcinoma cells. *Proc Am Assoc Cancer Res* 44:314, 2003

Struckhoff AP, Bittman R, Burow ME, Clejan S, Elliot S, Hammond T, Tang Y, **Beckman BS**. Novel ceramide analogs as potential chemotherapeutic agents in breast cancer. *Proc of the Am Assoc Cancer Res* 45:3746, 2004

Tang Y, Weldon CB, Elliott S, Butler NN, Alam J, **Beckman BS**, Curiel TJ, Burow ME. Effects of the MEK5-Erk5 signaling pathway on regulation of cell survival in breast carcinoma cells. *Proc Am Assoc Cancer Res* 44:1363, 2003

APPENDIX C.

USEABLE TECHNOLOGIES

Summary of Useable Technologies

Environmental Signals and Sensors

Blake, R.

The immediate objectives of this overall project have been to conduct detailed fundamental studies on the binding properties of three novel antibodies (designated as 1B11, 5B2, and 2D42) that are destined to be incorporated into the Autonomous Underwater Vehicle (AUV) as part of our ongoing developmental activities. The antibody-based biosensor will automatically collect and analyze 5 separate samples after installation in an autonomous underwater vehicle or immobilized buoy. A self-contained, automated immunosensor will have the capability to detect very low concentrations of environmental contaminants and/or chemical and biological weapons in surface waters. An assay that detects nanomolar levels of EDTA, the first analyte to be developed for this instrument, has been established. Transfer of the assay to the immunosensor will begin when Sapidyne has corrected the defects in the optical components of the instrument. Sapidyne Instruments (Boise, ID) is constructing the immunosensor with the Tulane laboratory. The approximate division of work is that the fundamental kinetic and thermodynamic studies are to be conducted at Xavier, while Dr. Diane Blake at Tulane conducts the more applied developmental studies.

McLachlan, JA

In vivo cell culture models have been established for the examination of gene expression relevant to genetic and possibly environmental contaminants. The cell systems can be utilized to screen differential gene expression. This type of screening would provide information as to potential deleterious effects of certain genetic and environmental chemicals or methodologies to classify these chemicals based upon gene expression profiles.

APPENDIX D.

INTELLECTUAL DEVELOPMENT

Intellectual Development

<u>Student Name</u>	<u>Level</u>	<u>Institution</u>	<u>Mentor</u>
Gutierrez-Figueroa, Yanira	Graduate	Tulane University	Barbara Beckman, Ph.D.
Struckhoff, Amanda P.	Graduate	Tulane University	Barbara Beckman, Ph.D.
Weldon, Christopher B.	Graduate	Tulane University	Barbara Beckman, Ph.D.
Martin, Melvenia	Graduate	Tulane University	John McLachlan, Ph.D.
Goldsmith, Dreama	Undergraduate	Xavier University	Robert Blake, Ph.D.
Borders, Wayne	Undergraduate	Xavier University	Robert Blake, Ph.D.
Bolden, Tiffany	Undergraduate	Xavier University	Robert Blake, Ph.D.
Jackson, Teresa	Undergraduate	Xavier University	Robert Blake, Ph.D.
Ross, Javay	Undergraduate	Xavier University	Shubha Kale-Ireland, Ph.D.
Yapi, Rodney	Undergraduate	Xavier University	Shubha Kale-Ireland, Ph.D.
Miller, Jenais	Undergraduate	Xavier University	Shubha Kale-Ireland, Ph.D.
Williams, Candice	Undergraduate	Xavier University	Tanya K. McKinney, Ph.D.
Clinton, Antoine	SPRITE	Tulane University	Diane Blake, Ph.D.
Dallas, Torijuan	SPRITE	Tulane University	Shanker Japa, Ph.D.
Hwang, Jennifer	SPRITE	Tulane University	Sanda Clejan, Ph.D.
Hunter, Christina	SPRITE	Tulane University	Sharon Isern, Ph.D.
Jupiter, Ryan	SPRITE	Tulane University	Michael Schurr, Ph.D.
Lee, Nicole	SPRITE	Tulane University	Thomas Wiese, Ph.D.

APPENDIX E.

HISTORICAL DOCUMENTS

- ❖ BAA
- ❖ Award/Modification Letter(s)
- ❖ Timeline
- ❖ SF 298 Cover Sheet

ONR BAA Announcement # 03-001

Published in FedBizOpps on 05 SEP 2002



BROAD AGENCY ANNOUNCEMENT (BAA)

INTRODUCTION:

This publication constitutes a Broad Agency Announcement (BAA) as contemplated in Federal Acquisition Regulation (FAR) 6.102(d)(2) and Department of Defense Grant and Agreement Regulations (DODGARS) 22.315. A formal Request for Proposals (RFP), solicitation, and/or additional information regarding this announcement will not be issued. This announcement will remain open for approximately one year from the date of publication or until replaced by a successor BAA. This announcement replaces ONR BAA # 02-001, dated 28 August 2001. Proposals may be submitted any time during this period.

The Office of Naval Research (ONR) will not issue paper copies of this announcement. The ONR reserves the right to select for award, all some or none of the proposals in response to this announcement. The ONR reserves the right to fund all, some or none of the proposals received under this BAA. ONR provides no funding for direct reimbursement of proposal development costs. Technical and cost proposals (or any other material) submitted in response to this BAA will not be returned. It is the policy of ONR to treat all proposals as sensitive competitive information and to disclose their contents only for the purposes of evaluation.

Awards may take the form of contracts, grants, cooperative agreements (CAs), or other transactions (OTs) Agreements. Therefore, proposals submitted as a result of this announcement may fall under the purview of either the Federal Acquisition Regulations (FAR) or the Department of Defense Grant and Agreement Regulations (DODGARS).

I. GENERAL INFORMATION

1. Agency Name -

Office of Naval Research
Ballston Centre, Tower One

800 N. Quincy Street
Arlington, VA 22217-5660

2. Research Opportunity Title -

Long Range Navy and Marine Corps Science & Technology

3. Program Name -

N/A

4. Research Opportunity Number -

BAA 03-001

5. Response Date -

This announcement will remain open until 30 September 2003 or until replaced by a successor BAA. Proposals may be submitted any time during this period.

6. Research Opportunity Description -

The Office of Naval Research (ONR) is interested in receiving proposals for Long-Range Science and Technology (S&T) Projects which offer potential for advancement and improvement of Navy and Marine Corps operations. Readers should note that this is an announcement to declare ONR's broad role in competitive funding of meritorious research across a spectrum of science and engineering disciplines.

Prior to preparing proposals, potential offerors are strongly encouraged to contact the ONR Program Officer (technical point of contact) whose program best matches the offeror's field of interest as listed in the Science and Technology section of the ONR Home Page accessible through World Wide Web at http://www.onr.navy.mil/sci_tech/ and for ONR's International Field Office (IFO) at <http://www.onrifo.navy.mil/>.

7. Point(s) of Contact -

Questions of a technical nature should be submitted to the ONR Program Officer whose program best matches the offeror's field of interest as listed in the Science and Technology section of the ONR Home Page specified above.

8. Instrument Type(s) -

It is anticipated that awards may take the form of contracts, grants, cooperative agreements, and other transaction agreements, as appropriate.

9. Catalog of Federal Domestic Assistance (CFDA) Numbers -

12.300

10. Catalog of Federal Domestic Assistance (CFDA) Titles -

Basic and Applied Scientific Research (DOD)

11. Other Information -

This announcement is restricted to work relating to basic and applied research and development not related to the development of a specific system or hardware procurement. Contracts and assistance instruments to be awarded under this BAA are for scientific study and experimentation directed towards advancing the state-of-the-art or increasing knowledge or understanding. This announcement **does not** cover technical, engineering and other types of R&D support services.

II. AWARD INFORMATION

The amount and period of performance of each selected proposal will vary depending on the research area and the technical approach to be pursued by the selected offeror.

III. ELIGIBILITY INFORMATION

All responsible sources from academia and industry may submit proposals under this BAA. Historically Black Colleges and Universities (HBCUs) and Minority Institutions (MIs) are encouraged to submit proposals and join others in submitting proposals. However, no portion of this BAA will be set aside for HBCU and MI participation.

IV. APPLICATION AND SUBMISSION INFORMATION

1. Application and Submission Process -

Pre-proposals or "White Papers" are frequently desired by ONR Program Officers. Offerors should consult the cognizant ONR Program Officer regarding the desirability of "White Paper" submissions.

2. General Information for Content and Format of White Papers/Full Proposals -

The proposals submitted under this BAA are expected to be unclassified. However, confidential/classified proposals are permitted. The proposal submissions will be protected from unauthorized disclosure in accordance with FAR 15.207, applicable law, and DoD/DoN regulations. Offerors are expected to appropriately mark each page of their submission that contains proprietary information.

Alternatives to the format and content identified below may be appropriate depending on the scope and nature of the proposed effort. Coordinate any alternative proposal formats and contents relating to white papers and technical proposals (Volume 1 of the full proposals) with the cognizant ONR Program Officer. Alternative formats and content may be directed by the ONR Program Officer or may result from Offerors' suggestions approved by the ONR Program Officer.

a. White Papers

White Paper Format

- Paper Size – 8.5 x 11 inch paper
- Margins – 1" inch
- Spacing – single or double-spaced
- Font – Times New Roman, 12 point
- Copies – one (1) original, appropriate number of hard copies as discussed with the cognizant Program Officer, and one electronic copy on a 3.5" Diskette or CD-ROM, (in Microsoft® Word or Excel 97 compatible or .PDF format).

White Paper Content

- **Cover Page:** The Cover Page shall be labeled "PROPOSAL WHITE PAPER," and shall include the BAA number, proposed title, technical points of contact, with telephone number, facsimile number, and e-mail address.
- **Technical Concept:** A description of the technology innovation and technical risk areas.

For Basic Research

- **Future Naval Relevance (where applicable):** A description of potential naval relevance and contributions of the effort to the agency's specific mission.

For Applied Research and Advanced Technology Development

- **Operational Naval Concept (where applicable):** A description of the project objectives, the concept of operation for the new capabilities to be delivered, and the expected operational performance improvements.
- **Operational Utility Assessment Plan (where applicable):** A plan for demonstrating and evaluating the operational effectiveness of the Offeror's proposed products or processes in field experiments and/or tests in a simulated environment.

b. Full Proposals

Full Proposal Format – Volume 1 - Technical and Volume 2 - Cost Proposal

- Paper Size – 8.5 x 11 inch paper
- Margins – 1” inch
- Spacing – single or double-spaced
- Font – Times New Roman, 12 point
- Discuss the number of page limit on Volume 1 with the cognizant Program Officer. There are no page limitations to Volume 2.
- Copies – one (1) original, appropriate number of hard copies as discussed with the cognizant Program Officer, and one electronic copy on a 3.5” Diskette or CD-ROM, (in Microsoft® Word or Excel 97 compatible or .PDF format).

Full Proposal Content

Volume 1: Technical Proposal

Each section of the Technical Proposal must start on a new page.

- **Cover Page:** This must include the words “Technical Proposal” and the following:
 - 1) BAA number;
 - 2) Title of Proposal;
 - 3) Identity of prime Offeror and complete list of subcontractors, if applicable;
 - 4) Technical contact (name, address, phone/fax, electronic mail address)
 - 5) Administrative/business contact (name, address, phone/fax, electronic mail address) and;
 - 6) Duration of effort (differentiate basic effort and options)
- **Table of Contents:**
- **Statement of Work:** A Statement of Work (SOW) clearly detailing the scope and objectives of the effort and the technical approach. It is anticipated that the proposed SOW will be incorporated as an attachment to the resultant award instrument. To this end, such proposals must include a severable self-standing SOW without any proprietary restrictions, which can be attached to the contract or agreement award. When options are contemplated, the SOW must clearly identify separate optional task areas. Similarly, the SOW must include a section which lists all proposed deliverables.

For Basic Research

- **Future Naval Relevance (where applicable):** A description of potential naval relevance and contributions of the effort to the agency's specific mission.

For Applied Research and Advanced Technology Development

- **Operational Naval Concept (where applicable):** A description of the project objectives, the concept of operation for the new capabilities to be delivered, and the expected operational performance improvements.
- **Operational Utility Assessment Plan (where applicable):** A plan for demonstrating and evaluating the operational effectiveness of the Offeror's proposed products or processes in field experiments and/or tests in a simulated environment.
- **Project Schedule and Milestones:** A summary of the schedule of events and milestones.
- **Assertion of Data Rights:** Include here a summary of any proprietary rights to pre-existing results, prototypes, or systems supporting and/or necessary for the use of the research, results, and/or prototype. Any rights made in other parts of the proposal that would impact the rights in this section must be cross-referenced. If there are proprietary rights, the Offeror must explain how these affect its ability to deliver subsystems and toolkits for integration. Additionally, Offerors must explain how the program goals are achievable in light of these proprietary and/or restrictive limitations. If there are no claims of proprietary rights in pre-existing data, this section shall consist of a statement to that effect.
- **Deliverables:** A detailed description of the results and products to be delivered. The SOW should include a summary listing of these deliverables.
- **Management Approach:** A discussion of the overall approach to the management of this effort, including brief discussions of the total organization; use of personnel; project/function/subcontractor relationships; government research interfaces; and planning, scheduling and control practice. Identify which personnel and subcontractors (if any) will be involved. Submit resumes/curriculum vitae for the key personnel identified. Include a description of the facilities that are required for the proposed effort with a description of any Government Furnished Equipment/Hardware/Software/Information required, by version and/or configuration.

VOLUME 2: Cost Proposal

The Cost Proposal shall consist of a cover page and two parts, Part 1 will provide a detailed cost breakdown of all costs by cost category by calendar/fiscal year and Part 2 will Cost breakdown by task/sub-task using the same task numbers in the Statement of Work. Options must be separately priced.

Cover Page: The use of the SF 1411 is optional. The words "Cost Proposal" should appear on the cover page in addition to the following information:

- BAA number (ONR BAA 03-001);
- Title of Proposal;
- Identity of prime Offeror and complete list of subcontractors/sub-recipients, if applicable;
- Technical contact (name, address, phone/fax, electronic mail address)
- Administrative/business contact (name, address, phone/fax, electronic mail address) and;
- Duration of effort (differentiate basic effort and options)
- Summary statement of proposed costs
- Cognizant DCAA and DCMA point of contact, address, phone/fax, electronic mail address (if readily available)

Part 1: Detailed breakdown of all costs by cost category by calendar/fiscal year (when options are contemplated, options must be separately identified and priced by calendar/fiscal year):

- Direct Labor – Individual labor category or person, with associated labor hours and unburdened direct labor rates;
- Indirect Costs – Fringe Benefits, Overhead, G&A, COM, etc. (Must show base amount and rate)
- Proposed contractor-acquired equipment such as computer hardware for proposed research projects should be specifically itemized with costs or estimated costs. An explanation of any estimating factors, including their derivation and application, shall be provided. Where possible, indicate purchasing method (competition, price comparison, market review, etc.);
- Travel – Number of trips, destinations, duration, etc;
- Subcontract – A cost proposal as detailed as the Offeror's cost proposal will be required to be submitted by the subcontractor. The subcontractor's cost proposal can be provided in a sealed envelope with the Offeror's cost proposal or will be requested from the subcontractor at a later date;
- Consultant – Provide consultant agreement or other document which verifies the proposed loaded daily/hourly rate;
- Materials – Specifically itemized by cost element. An explanation of any estimating factors, including their derivation and application, shall be provided. Where possible, indicate purchasing method (competition, price comparison, market review, etc.);
- Other Directs Costs and;
- Fee/Profit including fee percentage.

Part 2 : Cost breakdown by task/sub-task corresponding to the same task numbers in the Statement of Work. When options are contemplated, options must be separately identified and priced by task/sub-task corresponding to the same task numbers in the Statement of Work.

3. Significant Dates and Times -

This announcement will remain open for approximately one year from the date of publication or until replaced by a successor BAA. Proposals may be submitted anytime during this period.

4. Submission of Late Proposals –

N/A

5. Address for the Submission of White Papers, if applicable, and Full Proposals –

Office of Naval Research
Attn*: _____
ONR Department Code: _____
800 North Quincy Street
Arlington, VA 22217-5660

**Cognizant ONR Program Officer/Point of Contact*

NOTE: FULL PROPOSALS SENT BY FAX OR E-MAIL WILL NOT BE CONSIDERED.

V. EVALUATION INFORMATION

1. Evaluation Criteria –

Award decisions will be based on a competitive selection of proposals resulting from a scientific review. Evaluations will be conducted using the following evaluation criteria: (1) overall scientific and technical merits of the proposal; (2) potential naval relevance and contributions of the effort to the agency's specific mission; (3) the offeror's capabilities, related experience, facilities, techniques or unique combinations of these which are integral factors for achieving the proposal objectives; (4) the qualifications, capabilities and experience of the proposed Principal Investigator, team leader and key personnel who are critical in achieving the proposal objectives; and (5) the realism of the proposed cost and availability of funds.

For proposed awards to be made as contracts to large businesses, the socio-economic merits of each proposal will be evaluated based on the extent of the Offeror's commitment in providing meaningful subcontracting opportunities for small businesses,

HUBZone small businesses, small disadvantaged businesses, woman-owned small businesses, veteran-owned small businesses, service disabled veteran-owned small businesses, historically black colleges and universities, and minority institutions.

2. Evaluation Panel -

Technical and cost proposals submitted under this BAA will be protected from unauthorized disclosure in accordance with FAR 3.104-5 and 15.207. The cognizant program officer and other Government scientific experts will perform the evaluation of technical proposals. Cost proposals will be evaluated by Government business professionals. Restrictive notices notwithstanding, one or more support contractors may be utilized as subject-matter-expert technical consultants. Similarly, support contractors may be utilized to evaluate cost proposals. However, proposal selection and award decisions are solely the responsibility of Government personnel. Each support contractor's employee having access to technical and cost proposals submitted in response to this BAA will be required to sign a non-disclosure statement prior to receipt of any proposal submissions.

VI. AWARD ADMINISTRATION INFORMATION

1. Administrative Requirements –

- The North American Industry Classification System (NAICS) code – The North American Industry Classification System (NAICS) code for this announcement is 541710 with a small business size standard of 500 employees.
- CCR - Successful Offerors not already registered in the Central Contractor Registry (CCR) will be required to register in CCR prior to award of any grant, contract, cooperative agreement, or other transaction agreement. Information on CCR registration is available at <http://www.onr.navy.mil/02/ccr.htm>.
- Certifications – Proposals should be accompanied by a completed certification package which can be accessed on the ONR Home Page at Contracts & Grants. For grant proposals and proposals for cooperative agreements or other transaction agreements (other than for prototypes), the certification package is entitled, "Certifications for Grants and Agreements." For contract proposals and for other transaction proposals involving prototypes (Section 845 agreements), the certification package is entitled, "Representations and Certifications for Contracts."
- Subcontracting Plans - Successful contract proposals that exceed \$500,000, submitted by all but small business concerns, will be required to submit a Small Business Subcontracting Plan in accordance with FAR 52.219-9, prior to award.
- Models – Representative terms and conditions for cooperative agreements and other transaction agreements; and mandatory and discretionary FAR and DFARS

clauses for contract documents may be found on the ONR website at <http://www.onr.navy.mil/02/model.htm>

2. Reporting -

The following is a sample of deliverables that could be required under a typical research effort:

- Technical and Financial Progress Reports
- Presentation Material
- Other Documents or Reports
- Final Report

However, please note that specific deliverables (that may include software and hardware deliverables) may be proposed by each offeror and finalized during negotiations.

VII. OTHER INFORMATION

1. Government Property/Government Furnished Equipment (GFE) and Facilities

Each proposer must provide a very specific description of any equipment/hardware that it needs to acquire to perform the work. This description should indicate whether or not each particular piece of equipment/hardware will be included as part of a deliverable item under the resulting award. Also, this description should identify the component, nomenclature, and configuration of the equipment/hardware that it proposes to purchase for this effort. It is the Government's desire to have the contractors purchase the equipment/hardware for deliverable items under their contract. The purchase on a direct reimbursement basis of special test equipment or other equipment that is not included in a deliverable item will be evaluated for allowability on a case-by-case basis. Maximum use of Government integration, test, and experiment facilities is encouraged in each of the Offeror's proposals.

Government research facilities and operational military units are available and should be considered as potential government furnished equipment/facilities. These facilities and resources are of high value and some are in constant demand by multiple programs. It is unlikely that all facilities would be used for any one specific program. The use of these facilities and resources will be negotiated as the program unfolds. Offerors should explain which of these facilities they recommend.

2. Security Classification

In order to facilitate intra-program collaboration and technology transfer, the Government will attempt to enable technology developers to work at the unclassified level to the

maximum extent possible. If access to classified material will be required at any point during performance, the offeror must clearly identify such need prominently in their proposal.

If developers use unclassified data in their deliveries and demonstrations regarding a potential classified project, they should use methods and conventions consistent with those used in classified environments. Such conventions will permit the various subsystems and the final system to be more adaptable in accommodating classified data in the transition system.


3. Use of Animals and Human Subjects in Research

If animals are to be utilized in the research effort proposed, the Offeror must complete a DoD Animal Use Protocol with supporting documentation (copies of AAALAC accreditation and /or NIH assurance, IACUC approval, research literature database searches, and the two most recent USDA inspection reports) prior to award. Similarly, for any proposal that involves the experimental use of human subjects, the Offeror must obtain approval from the Offeror's committee for protection of human subjects (normally referred to as an Institutional Review Board, (IRB)). The Offeror must also provide NIH (OHRP/DHHS) documentation of a Federal Wide Assurance that covers the proposed human subjects study. If the Offeror does not have a Federal Wide Assurance, a DoD Single Project Assurance for that work must be completed prior to award. Please see <http://www.onr.navy.mi./02/howto.htm> for further information.

3. Department of Defense High Performance Computing Program

The DoD High Performance Computing Program (HPCMP) furnishes the DoD S & T and DT & E communities with use-access to very powerful high performance computing systems. Awardees of ONR contracts, grants, and assistance instruments may be eligible to use HPCMP assets in support of their funded activities if ONR Program Officer approval is obtained and if security/screening requirements are favorably completed. Additional information and an application may be found at <http://www.hpcmo.hpc.mil/>.

05/04

		<h1 style="text-align: center;">AWARD/ MODIFICATION</h1>		3a. ISSUED BY: OFFICE OF NAVAL RESEARCH BALLSTON CENTRE TOWER ONE 800 NORTH QUINCY STREET ARLINGTON VA 22217-5660	
				3b. CFDA: 12.300	
1. INSTRUMENT TYPE: Grant		2. AUTHORITY: 10 USC 2358, 31 USC 6304		3c. DUNS NUMBER:	
4. AWARD NO.: N00014-99-1-0763		5. MODIFICATION NO.: P00006		6. MODIFICATION TYPE: Renewal	
8. ACTIVITY/AGENCY PROPOSAL NO.: 03342--0110		9. RECIPIENT PROPOSAL NO.: N/A		10. PROPOSAL DATE: 22-JAN-2003	
11. ACTIVITY TYPE: Research		12. PROGRAM TYPE: N/A		7. PR NO.: 03PR08048-00	
13. ISSUED TO 13a. ADDRESS: TULANE UNIVERSITY OFFICE OF RESEARCH AND PROJECT ADMINISTRATION 7029 FRERET STREET NEW ORLEANS, LA 70118-5698		13b. CAGE: 4B966		13c. EDI/EFT NUMBER: 2142AL	
13d. BUSINESS OFFICE CONTACT: Kozar, Kathleen		13e. TELEPHONE NUMBER: (504) 5885207		13f. EMAIL ADDRESS: kkozar@mailhost.tcs.tulane.edu	
14. REMITTANCE ADDRESS (IF DIFFERENT FROM BLOCK 13): Same as block #13					
15. RESEARCH TITLE AND/OR DESCRIPTION OF PROJECT AND/OR PROPOSAL TITLE: Integrated Bioenvironmental Hazards Research Program					
16. FUNDING		ACTIVITY/AGENCY SHARE		RECIPIENT SHARE	
PREVIOUSLY OBLIGATED:		\$8,475,500.00		\$0.00	
OBLIGATED BY THIS ACTION:		\$1,100,000.00		\$0.00	
TOTAL OBLIGATED ON AWARD:		\$9,575,500.00		\$0.00	
FUTURE FUNDING:		\$0.00		\$0.00	
GRANT TOTAL:		\$9,575,500.00		\$0.00	
TOTAL:		\$8,475,500.00		\$8,475,500.00	
TOTAL:		\$1,100,000.00		\$1,100,000.00	
TOTAL:		\$9,575,500.00		\$9,575,500.00	
TOTAL:		\$0.00		\$0.00	
TOTAL:		\$9,575,500.00		\$9,575,500.00	
17. CURRENT FUNDING PERIOD N/A THROUGH N/A					
18. PERIOD OF PERFORMANCE 01-MAY-1999 THROUGH 30-JUN-2004					
19. ACCOUNTING AND APPROPRIATION DATA: See attached Financial Accounting Data Sheet(s)					
20a. PRINCIPAL INVESTIGATOR/RECIPIENT TECHNICAL REPRESENTATIVE: (PI) John McLachlan		21. TECHNICAL REPRESENTATIVE 21a. NAME: Joe L. Brumfield		21b. CODE: ONR 342	
20b. TELEPHONE NUMBER: (504) 5856910		20c. EMAIL ADDRESS:		21c. ADDRESS: OFFICE OF NAVAL RESEARCH BALLSTON CENTRE TOWER ONE 800 NORTH QUINCY STREET ARLINGTON VA 22217-5660	
22. AWARDING OFFICE CONTACT 22a. NAME: Julia M. Gallmon		22b. CODE: ONR 252		21d. TELEPHONE NUMBER: (703) 6964057	
22c. ADDRESS: OFFICE OF NAVAL RESEARCH BALLSTON CENTRE TOWER ONE 800 NORTH QUINCY STREET ARLINGTON VA 22217-5660		22d. TELEPHONE NUMBER: (703) 6962609		21e. EMAIL ADDRESS: brumfi@onr.navy.mil	
22e. EMAIL ADDRESS: gallmoj@onr.navy.mil		23a. ADMINISTRATIVE OFFICE: OFFICE OF NAVAL RESEARCH REGIONAL OFFICE ATLANTA 100 ALABAMA STREET SW SUITE 4R15 ATLANTA GA 30303-3104 Fax: (404) 5621610		23b. CODE: N66020	
24. SUBMIT PAYMENT REQUEST TO: Same as block #23a		25a. PAYING OFFICE: DFAS CHARLESTON, SC		25b. CODE: N68892	
26a. PATENT OFFICE: OFFICE OF NAVAL RESEARCH ATTN: ONR 00CC BALLSTON CENTRE TOWER ONE 800 NORTH QUINCY STREET ARLINGTON VA 22217-5660		26b. CODE: N00014			

AWARD NO. N00014-99-1-0763		AWARD/MODIFICATION		MODIFICATION NO. P00006		PAGE 2 of 5	
27. SPECIAL INSTRUCTIONS: See "Special Requirements" Attachment							
28. DELEGATIONS: The administration duties listed below have been delegated to the administrative office (block 23a). Upon request the awarding office contact (block 22) will make their full text available. Please direct questions to the contacts @: http://www.onr.navy.mil/02/024/offices.htm							
Full Delegation							
29. TERMS AND CONDITIONS: The following terms and conditions are incorporated herein by reference with the same force and effect as if they were given in full text. Upon request the awarding office contact named in block 22 will make their full text available, or they can be found at the specified URL.							
DOCUMENT		URL				CLAUSES	
UAWA Award A		http://www.antd.nist.gov/fededi/resources/documents/					
UAAC Acceptance C		http://www.antd.nist.gov/fededi/resources/documents/					
UBB1 FDP IV October 2002		http://www.nsf.gov/home/grants/grants_fdp.htm					
UVV1 ONR PDP Specific OCT 2002		http://www.nsf.gov/pubs/fdp/onr02.pdf					
30. OPTIONS							
OPTION NO.		AMOUNT		PERIOD			
(1)							
(2)							
(3)							
(4)							
31. REPORTS: The following reports must be submitted to the indicated addressees, in the indicated quantities, within 90 days following the expiration or termination of the project. Final Technical Reports must have a SF298, Report Documentation Page, accompanying them. Unless otherwise stated in the award/modification, complete Block 12a of the SF298 as follows: "Approved for Public Release; distribution is Unlimited".							
ADDRESSEE		REPORT TYPE				COPIES	
See block #21 (Frequency)		Final Technical Report w/ SF298				1	
		Performance/Technical Report (As Required) w/ SF298				1	
See block #23a		Report of Inventions and Subcontracts - DD 882				1	
		Final Technical Report - Transmittal letter only				1	
		Performance/Technical Report (As Required)				1	
		Final Financial Status Report - SF269A - If advances used				1	
See block #26a		Report of Inventions and Subcontracts - DD 882				1	
DEFENSE TECHNICAL INFORMATION CENTER 8725 JOHN J KINGMAN ROAD STE 0944 FORT BELVOIR VA 22060-6218		Final Technical Report w/ SF298				1	
		Performance/Technical Report (As Required) w/ SF298				1	
NAVAL RESEARCH LABORATORY ATTN: CODE 5227 4555 OVERLOOK AVENUE SW WASHINGTON DC 20375-5320		Final Technical Report w/ SF298				1	
		Performance/Technical Report (As Required) w/ SF298				1	
32. FOR THE RECIPIENT				33. FOR THE UNITED STATES OF AMERICA			
32a. SIGNATURE OF PERSON AUTHORIZED TO SIGN N/A - SIGNATURE NOT REQUIRED ON THIS AWARD				33a. SIGNATURE OF AWARDOFFICER <i>R. Brian Bradley</i>			
32b. NAME AND TITLE OF SIGNER		32c. DATE SIGNED		33b. NAME AND TITLE OF AWARDOFFICER R B. Bradley		33c. DATE SIGNED 25-FEB-2003	

ONR 2003 - 2004 Timeline

 $\leq \leq \leq$ Research
Support

Core
Support

Environmental Signals and Sensors

Environmental Monitoring and Assessment

07 / 2003

06 / 2004

Tulane Research Support

Xavier Research Support

Computer Operations

Environmental Informatics

Communication and Education

07 / 03

Johanning, R. Blake, Kale-Ireland, McKinney, Beckman, McLachlan

Meffert

Jul 2003	Aug 2003	Sept 2003	Oct 2003	Nov 2003	Dec 2003	Jan 2004	Feb 2004	Mar 2004	Apr 2004	May 2004	Jun 2004
----------	----------	-----------	----------	----------	----------	----------	----------	----------	----------	----------	----------

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE (DD-MM-YYYY) 09-02-2005		2. REPORT TYPE Performance Technical Report		3. DATES COVERED (From - To) July 2003 - June 2004	
4. TITLE AND SUBTITLE Integrated Bioenvironmental Hazards Program				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER N000014-99-1-0763	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) McLachlan, John Dr., PI Meffert, Douglas, Dr., Deputy Director Kitzman, Helen, Dr., Project Administrator Johnson, Desiree, Program Manager Maag, Dave, Microsystem Analyst				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Tulane University 1430 Tulane Avenue, sl-3 New Orleans, LA 70112				8. PERFORMING ORGANIZATION REPORT NUMBER Xavier University of Louisiana 1 Drexel Drive New Orleans, LA 70118	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Office of Naval Research Ballston Centre Tower One 800 North Quincy Street Arlington, VA 22217-5660				10. SPONSOR/MONITOR'S ACRONYM(S) ONR	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT APPROVED FOR PUBLIC RELEASE					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Since April 1999, the Center for Bioenvironmental Research (CBR) at Tulane and Xavier Universities has received funding from the Office of Naval Research (ONR) to continue its Bioenvironmental Hazards Research Program (BHRP). This funding has supported a suite of complementary research projects that address the impacts of bioenvironmental hazards on environmental signaling from molecular to ecosystem levels and make connections between these impacts. One module, Environmental Signals and Sensors, utilizes basic research on how chemical signals at molecular, cellular, and organismal levels can be utilized for assessments of human, wildlife, and plant health. A second module, Ecosystem Monitoring, emphasizes research on small scale turbulence and the development of biosensors and autonomous platforms for assessments of toxicity and risk. The BHRP program includes mechanisms for effective communication of this information for resolution of DOD problems and for educational training of future scientists. Transcending traditional structures, the CBR has become a model of academic/government/industry interaction.					
15. SUBJECT TERMS Autonomous Underwater Vehicles (AUV), Biosensors, Environmental Signals & Sensors, Communication & Education					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT SAR	18. NUMBER OF PAGES 73	19a. NAME OF RESPONSIBLE PERSON Dr. Douglas Meffert, Deputy Director
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (Include area code) (504) 988-6910